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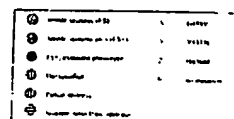
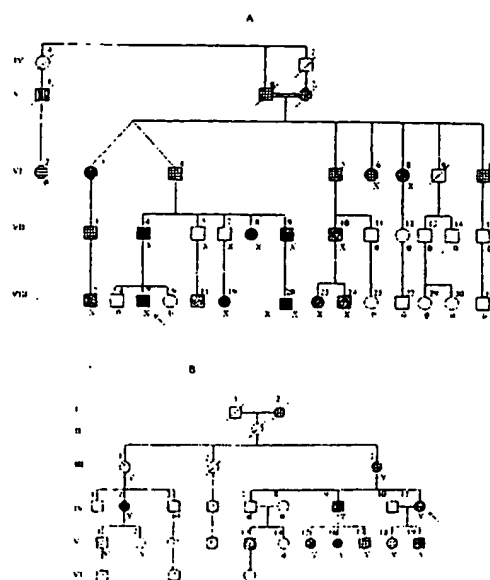
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(54) Title: **SODIUM-CHANNEL ALPHA1-SUBUNIT AND THEIR POLYPEPTIDES AND THEIR TREATMENT OF GENERALISED EPILEPSY WITH FEBRILE SEIZURES PLUS**



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(57) Abstract: The mutations D188V, V1353L, I1656M in the neuronal gene sodium-channel alpha1-subunit, SCN1A, are disclosed. The methods of using their associated polypeptides for treating sodium channel dysfunction disorders including generalised epilepsy are also disclosed.



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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Mutations in Neuronal gene sodium-channel alpha1-subunit and their polypeptides and their treatment of generalised epilepsy with febrile seizures plus.

Technical Field

The present invention relates to mutations in the alpha subunit of mammalian voltage-gated sodium channels which are associated with idiopathic epilepsies and other disorders such as malignant hyperthermia, myasthenia, episodic ataxia, neuropathic and inflammatory pain, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, myotonias and cardiac arrhythmias, and to polymorphisms in the gene encoding the alpha subunit.

Background Art

Generalised epilepsy with febrile seizures plus (GEFS+; MIM 604236) was first described by Scheffer and Berkovic (1997) and is now recognised as a common epilepsy syndrome (Singh et al. 1999; Baulac et al. 1999; Moulard et al. 1999; Peiffer et al. 1999; Scheffer et al. 2000). Although GEFS+ is familial, it was initially difficult to recognise it as a distinct syndrome, because of clinical heterogeneity within each family. The common phenotypes are typical febrile seizures (FS) and febrile seizures plus (FS+); FS+ differs from FS in that the attacks with fever continue beyond age 6 years and/or include afebrile tonic-clonic seizures. Less common phenotypes include FS+ associated with absences, myoclonic or atonic seizures, and even more-severe syndromes such as myoclonic-astatic epilepsy. That such phenotypic diversity could be associated with the segregation of a mutation in a single gene was established with the identification of a mutation in the voltage gated sodium channel beta-1 subunit gene (SCN1B) (Wallace et al. 1998). This mutation (C121W) changes a conserved cysteine residue, disrupting a putative disulfide bridge, which results in in vitro loss of function of the beta-1 subunit. Without a functional beta-1 subunit the rate of inactivation of sodium channel alpha subunits decreases, which may cause increased sodium

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influx, resulting in a more depolarised membrane potential and hyperexcitability. Modifier genes or the environment may interact with the SCN1B gene to account for clinical heterogeneity, but the rarity of SCN1B mutations (Wallace et al. 1998) strongly suggested additional genes of large effect underlie GEFS+ in other families (Singh et al. 1999).

GEFS+ in four families has been mapped to chromosome 2q (Baulac et al. 1999; Moulard et al. 1999; Peiffer et al. 1999; Lopes-Cendes et al. 2000). Recently, mutations in the neuronal voltage gated sodium channel alpha-1 (SCN1A) subunit were described in two GEFS+ families (Escayg et al. 2000). The mutations (T875M and R1648H) are located in highly conserved S4 transmembrane segments of the channel which are known to have a role in channel gating. It was suggested that these mutations may reduce the rate of inactivation of SCN1A and therefore have a similar effect as the beta-1 subunit mutation.

GEFS+ is clearly a common complex disorder, with a strong genetic basis, incomplete penetrance and genetic and phenotypic heterogeneity. Febrile seizures occur in 3% of the population, and thus this phenotype may occur sporadically in GEFS+ families, in addition to occurring as a result of an inherited mutation in the GEFS+ gene (Wallace et al 1998). Also, although some families segregate an autosomal dominant gene of major effect, in many cases clinical genetic evidence, such as bilineality, suggests that for some small families the disorder is multifactorial (Singh et al 1999). Despite this, large families continue to be ascertained and with critical phenotypic analysis, they provide opportunities to localise and ultimately identify the genes involved.

Disclosure of the Invention

The present inventors have identified three new mutations in the alpha-1 subunit (SCN1A) of the voltage-gated sodium channel that are associated with epilepsy, in

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particular generalized epilepsy with febrile seizures plus (GEFS+), and also determined the nucleotide sequence in that gene.

According to one aspect of the present invention
5 there is provided an isolated DNA molecule encoding a mutant alpha subunit of a mammalian voltage-gated sodium channel, wherein a mutation event selected from the group consisting of point mutations, deletions, insertions and rearrangements has occurred and said mutation event
10 disrupts the functioning of an assembled sodium channel so as to produce an epilepsy phenotype, with the proviso that the mutation event is not a C2624T transition or a G4943A transition in an alpha-1 subunit.

Preferably said mutation event is a point mutation.

15 Typically the mutation event occurs in an intracellular loop, preferably in the intracellular loop between transmembrane segments 2 and 3 of domain I, in the S4 segment of domain IV at amino acid position 1656, or in an S5 segment of a transmembrane domain. Preferably the
20 mutation creates a phenotype of generalised epilepsy with febrile seizures plus.

In one form of the invention the mutation is in exon
4 of SCN1A and results in replacement of a highly conserved aspartic acid residue with a valine residue at
25 amino acid position 188. The D188V mutation lies in the intracellular loop just outside the S3 segment of domain I of SCN1A and occurs as a result of an A to T nucleotide substitution at position 563 of the SCN1A coding sequence as shown in SEQ ID NO:1.

30 In a further form of the invention the mutation is in exon 21 of SCN1A and results in the replacement of a highly conserved valine residue with a leucine residue at amino acid position 1353. The V1353L mutation is located in the S5 segment of domain III of SCN1A and occurs as a
35 result of a G to C nucleotide substitution at position 4057 of the SCN1A coding sequence as shown in SEQ ID NO:3.

In a still further form of the invention the mutation

is in exon 26 of SCN1A and results in the replacement of a highly conserved isoleucine residue with a methionine residue at amino acid position 1656. The I1656M mutation is located in the S4 segment of domain IV of SCN1A and
5 occurs as a result of a C to G nucleotide substitution at position 4968 of the SCN1A coding sequence as shown in SEQ ID NO:5.

The nucleotide sequence of the gene set forth in SEQ ID NO:89 also forms a part of the invention. In addition,
10 the polymorphisms identified in Table 3 form part of the invention (SEQ ID Numbers:7-9 and 11).

The present invention also encompasses DNA molecules in which one or more additional mutation events selected from the group consisting of point mutations, deletions,
15 insertions and rearrangements have occurred. Any such DNA molecule will have the mutation associated with epilepsy described above and will be functional, but otherwise may vary significantly from the DNA molecules set forth in SEQ ID NO:1, 3 and 5.

The nucleotide sequences of the present invention can be engineered using methods accepted in the art for a variety of purposes. These include, but are not limited to, modification of the cloning, processing, and/or expression of the gene product. PCR reassembly of gene
25 fragments and the use of synthetic oligonucleotides allow the engineering of the nucleotide sequences of the present invention. For example, oligonucleotide-mediated site-directed mutagenesis can introduce further mutations that create new restriction sites, alter expression patterns
30 and produce splice variants etc.

As a result of the degeneracy of the genetic code, a number of polynucleotide sequences, some that may have minimal similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus,
35 the invention includes each and every possible variation of a polynucleotide sequence that could be made by selecting combinations based on possible codon choices.

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These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequences of the present invention, and all such variations are to be considered as being specifically disclosed.

The DNA molecules of this invention include cDNA, genomic DNA, synthetic forms, and mixed polymers, both sense and antisense strands, and may be chemically or biochemically modified, or may contain non-natural or derivatised nucleotide bases as will be appreciated by those skilled in the art. Such modifications include labels, methylation, intercalators, alkylators and modified linkages. In some instances it may be advantageous to produce nucleotide sequences possessing a substantially different codon usage than that of the polynucleotide sequences of the present invention. For example, codons may be selected to increase the rate of expression of the peptide in a particular prokaryotic or eukaryotic host corresponding with the frequency that particular codons are utilized by the host. Other reasons to alter the nucleotide sequence without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring mutated sequence.

The invention also encompasses production of DNA sequences of the present invention entirely by synthetic chemistry. Synthetic sequences may be inserted into expression vectors and cell systems that contain the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. These elements may include regulatory sequences, promoters, 5' and 3' untranslated regions and specific initiation signals (such as an ATG initiation codon and Kozak consensus sequence) which allow more efficient translation of sequences encoding the polypeptides of the present invention. In cases where the complete coding

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sequence, including the initiation codon and upstream regulatory sequences, are inserted into the appropriate expression vector, additional control signals may not be needed. However, in cases where only coding sequence, or a
5 fragment thereof, is inserted, exogenous translational control signals as described above should be provided by the vector. Such signals may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the
10 particular host cell system used (Scharf et al., 1994).

The invention also includes nucleic acid molecules that are the complements of the sequences described herein.

According to still another aspect of the present
15 invention there is provided an isolated DNA molecule consisting of the nucleotide sequence set forth in any one of SEQ ID NOS:1, 3, 5, 7, 8, 9, 11 and 89.

The present invention allows for the preparation of purified polypeptides or proteins from the polynucleotides
20 of the present invention, or variants thereof. In order to do this, host cells may be transformed with a DNA molecule as described above. Typically said host cells are transfected with an expression vector comprising a DNA molecule according to the invention. A variety of
25 expression vector/host systems may be utilized to contain and express sequences encoding polypeptides of the invention. These include, but are not limited to, microorganisms such as bacteria transformed with plasmid or cosmid DNA expression vectors; yeast transformed with
30 yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); or mouse or other animal or human tissue cell systems. Mammalian cells can be used to express a protein using various expression vectors including plasmid, cosmid and
35 viral systems such as a vaccinia virus expression system. The invention is not limited by the host cell employed.

The polynucleotide sequences, or variants thereof, of

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the present invention can be stably expressed in cell lines to allow long term production of recombinant proteins in mammalian systems. Sequences encoding the polypeptides of the present invention can be transformed
5 into cell lines using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. The selectable marker confers resistance to a selective agent, and its presence allows growth and
10 recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

The protein produced by a transformed cell may be
15 secreted or retained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode a protein may be designed to contain signal sequences which direct secretion of the
20 protein through a prokaryotic or eukaryotic cell membrane.

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include,
25 but are not limited to, acetylation, glycosylation, phosphorylation, and acylation. Post-translational cleavage of a "prepro" form of the protein may also be used to specify protein targeting, folding, and/or activity. Different host cells having specific cellular
30 machinery and characteristic mechanisms for post-translational activities (e.g., CHO or HeLa cells), are available from the American Type Culture Collection (ATCC) and may be chosen to ensure the correct modification and processing of the foreign protein.

35 When large quantities of the gene are needed, such as for antibody production, vectors which direct high levels of expression of this protein may be used, such as those

containing the T5 or T7 inducible bacteriophage promoter. The present invention also includes the use of the expression systems described above in generating and isolating fusion proteins which contain important functional domains of the protein. These fusion proteins are used for binding, structural and functional studies as well as for the generation of appropriate antibodies.

In order to express and purify the protein as a fusion protein, the appropriate polynucleotide sequences of the present invention are inserted into a vector which contains a nucleotide sequence encoding another peptide (for example, glutathione-s-transferase). The fusion protein is expressed and recovered from prokaryotic or eukaryotic cells. The fusion protein can then be purified by affinity chromatography based upon the fusion vector sequence. The desired protein is then obtained by enzymatic cleavage of the fusion protein.

Fragments of polypeptides of the present invention may also be produced by direct peptide synthesis using solid-phase techniques. Automated synthesis may be achieved by using the ABI 431A Peptide Synthesizer (Perkin-Elmer). Various fragments of this protein may be synthesized separately and then combined to produce the full length molecule.

According to still another aspect of the present invention there is provided an isolated polypeptide, said polypeptide being a mutant alpha subunit of a mammalian voltage-gated sodium channel, wherein a mutation event selected from the group consisting of point mutations, deletions, insertions and rearrangements has occurred and said mutation event disrupts the functioning of an assembled sodium channel so as to produce an epilepsy phenotype, with the proviso that said mutation event is not a T875M transition or a R1648H transition in an alpha-1 subunit.

Preferably said mutation event occurs in an intracellular loop, preferably in the intracellular loop

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between transmembrane segments 2 and 3 in domain I, in the S4 segment of domain IV at amino acid position 1656, or in an S5 segment of a transmembrane domain of SCN1A. Preferably the mutation creates a phenotype of generalised epilepsy with febrile seizures plus.

In one form of the invention the mutation event is a substitution in which a highly conserved aspartic acid residue is replaced with a valine residue located in the intracellular domain located just outside the S3 segment of domain I of SCN1A. Preferably the substitution is a D188V transition as illustrated in SEQ ID NO:2.

In a further form of the invention the mutation event is a substitution in which a highly conserved valine residue is replaced with a leucine residue located in the S5 segment of domain III of SCN1A. Preferably the substitution is a V1353L transition as illustrated in SEQ ID NO:4.

In a still further form of the invention the mutation event is a substitution in which a highly conserved isoleucine residue is replaced with a methionine residue located in the S4 segment of domain IV of SCN1A. Preferably the substitution is a I1656M transition as illustrated in SEQ ID NO:6.

In addition, the polymorphisms identified in Table 3 form part of the invention (SEQ ID Numbers:10 and 12). These polymorphisms may reflect changes in SCN1A which result in subtle changes of function of the sodium channel. These subtle changes may predispose individuals to epilepsy and when expressed in combination with other ion channel changes may lead to specific sub-types of the disease (see PCT/AU01/00872).

The isolated polypeptides of the present invention may have been subjected to one or more mutation events selected from the group consisting of substitutions, deletions, insertions and rearrangements in addition to the mutation associated with epilepsy. Typically these mutation events are conservative substitutions.

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According to still another aspect of the present invention there is provided an isolated polypeptide comprising the sequence set forth in any one of SEQ ID NO:2, 4, 6, 10 and 12.

5 According to still another aspect of the present invention there is provided a polypeptide consisting of the amino acid sequence set forth in any one of SEQ ID NO:2, 4, 6, 10 and 12.

10 According to still another aspect of the present invention there is provided an isolated polypeptide complex, said polypeptide complex being an assembled mammalian voltage-gated sodium channel, wherein a mutation event selected from the group consisting of substitutions, deletions, insertions and rearrangements has occurred in
15 the alpha subunit of the complex. Mutations include those in the intracellular loop between transmembrane segments 2 and 3, the S4 segment of domain IV at amino acid position 1656, or in an S5 segment of a transmembrane domain of the
20 mammalian voltage-gated sodium channel bearing any such mutation in the alpha subunit will produce a phenotype of epilepsy, in particular generalised epilepsy with febrile seizures plus, or other disorders associated with sodium channel dysfunction including, but not restricted to,
25 malignant hyperthermia, myasthenia, episodic ataxia, neuropathic and inflammatory pain, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, myotonias such as hypo- and hyperkalaemic periodic paralysis, paramyotonia congenita and potassium aggravated
30 myotonia as well as cardiac arrhythmias such as long QT syndrome.

In a particular aspect there is provided a complex, being an assembled mammalian voltage-gated sodium channel, bearing a mutation in the intracellular loop between
35 transmembrane segments 2 and 3, the S4 segment of domain IV at amino acid position 1656, or in an S5 segment of a transmembrane domain of the SCN1A subunit of the channel.

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According to still another aspect of the present invention there is provided a method of preparing a polypeptide, said polypeptide being a mutant alpha subunit of a mammalian voltage-gated sodium channel, comprising the steps of:

- (1) culturing host cells transfected with an expression vector comprising a nucleic acid molecule as described above under conditions effective for polypeptide production; and
- (2) harvesting the mutant alpha subunit.

The mutant alpha subunit may also be allowed to assemble with other subunits of the sodium channel, whereby the assembled mutant sodium channel is harvested.

According to still another aspect of the invention there is provided a polypeptide which is the product of the process described above.

Substantially purified protein or fragments thereof can then be used in further biochemical analyses to establish secondary and tertiary structure for example by X-ray crystallography of crystals of the proteins or by nuclear magnetic resonance (NMR). Determination of structure allows for the rational design of pharmaceuticals to interact with the mutated sodium channel, alter the overall sodium channel protein charge configuration or charge interaction with other proteins, or to alter its function in the cell.

It will be appreciated that, having identified mutations involved in epilepsy in these proteins, the mutant sodium channel alpha subunits will be useful in further applications which include a variety of hybridisation and immunological assays to screen for and detect the presence of either a normal or mutated gene or gene product. The invention also enables therapeutic methods for the treatment of epilepsy and enables methods for the diagnosis of epilepsy with both wild-type and mutant nucleic acid molecules. In particular the invention enables treatment and diagnosis of generalised epilepsy

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with febrile seizures plus, as well as other disorders associated with sodium channel dysfunction, as mentioned above.

5 Therapeutic Applications

According to one aspect of the invention there is provided a method of treating epilepsy, in particular generalised epilepsy with febrile seizures plus, as well as other disorders associated with sodium channel
10 dysfunction, including but not restricted to, malignant hyperthermia, myasthenia, episodic ataxia, neuropathic and inflammatory pain, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, myotonias such as hypo- and hyperkalaemic periodic paralysis, paramyotonia
15 congenita and potassium aggravated myotonia as well as cardiac arrhythmias such as long QT syndrome, comprising administering a selective antagonist, agonist or modulator of the sodium channel when a mutation event as described above has occurred, in particular, when it contains a
20 mutation in the intracellular loop between transmembrane segments 2 and 3, in the S4 segment of domain IV at amino acid position 1656, or in an S5 segment of a transmembrane domain of an alpha subunit.

In still another aspect of the invention there is
25 provided the use of a selective antagonist, agonist or modulator of the sodium channel when a mutation event as described above has occurred, in particular, to a sodium channel when it contains a mutation in the intracellular loop between transmembrane segments 2 and 3, in the S4
30 segment of domain IV at amino acid position 1656, or in an S5 segment of a transmembrane domain of an alpha subunit, said mutation being causative of a disorder including epilepsy, in particular generalised epilepsy with febrile seizures plus as well as other disorders associated with
35 sodium channel dysfunction, including but not restricted to, malignant hyperthermia, myasthenia, episodic ataxia, neuropathic and inflammatory pain, Alzheimer's disease,

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Parkinson's disease, schizophrenia, hyperekplexia, myotonias such as hypo- and hyperkalaemic periodic paralysis, paramyotonia congenita and potassium aggravated myotonia as well as cardiac arrhythmias such as long QT syndrome, in the manufacture of a medicament for the treatment of the disorder.

In one aspect of the invention a suitable antagonist or modulator will restore wild-type function to the sodium channels that contain a mutation in an alpha subunit including those that form part of this invention.

Using methods well known in the art, a mutant sodium channel may be used to produce antibodies specific for the mutant channel that is causative of the disease or to screen libraries of pharmaceutical agents to identify those that specifically bind the mutant sodium channel.

In one aspect, an antibody, which specifically binds to a mutant sodium channel, may be used directly as an antagonist or modulator, or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissues that express the mutant sodium channel.

In a still further aspect of the invention there is provided an antibody which is immunologically reactive with a polypeptide as described above, but not with a wild-type sodium channel or subunit thereof.

In particular, there is provided an antibody to an assembled sodium channel containing a mutation causative of a disorder as described above, in a subunit comprising the receptor. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies as would be understood by the person skilled in the art.

For the production of antibodies, various hosts including rabbits, rats, goats, mice, humans, and others may be immunized by injection with a polypeptide as described or with any fragment or oligopeptide thereof which has immunogenic properties. Various adjuvants may be used to increase immunological response and include, but

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are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface-active substances such as lysolecithin. Adjuvants used in humans include BCG (bacilli Calmette-Guerin) and *Corynebacterium parvum*.

5 It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to the mutant sodium channel have an amino acid sequence consisting of at least 5 amino acids, and, more preferably, of at least 10 amino acids. It is also preferable that these oligopeptides,
10 peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein and contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of sodium channel amino acids may be fused with those of another protein, such as KLH,
15 and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to a mutant sodium channel may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the
20 hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (For example, see Kohler et al., 1975; Kozbor et al., 1985; Cote et al., 1983; Cole et al., 1984).

Antibodies may also be produced by inducing in vivo
25 production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature. (For example, see Orlandi et al., 1989; Winter et al., 1991).

Antibody fragments which contain specific binding
30 sites for a mutant sodium channel may also be generated. For example, such fragments include, F(ab')₂ fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab expression
35 libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (For example, see Huse et al., 1989).

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Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between a sodium channel and its specific antibody. A two-site, monoclonal-based immunoassay utilizing antibodies reactive to two non-interfering sodium channel epitopes is preferred, but a competitive binding assay may also be employed.

In a further aspect of the invention there is provided a method of treating epilepsy, in particular generalised epilepsy with febrile seizures plus, as well as other disorders associated with sodium channel dysfunction, including but not restricted to, malignant hyperthermia, myasthenia, episodic ataxia, neuropathic and inflammatory pain, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, myotonias such as hypo- and hyperkalaemic periodic paralysis, paramyotonia congenita and potassium aggravated myotonia as well as cardiac arrhythmias such as long QT syndrome, comprising administering an isolated DNA molecule which is the complement (antisense) of any one of the DNA molecules described above and which encodes an RNA molecule that hybridizes with the mRNA encoding a mutant sodium channel alpha subunit, to a subject in need of such treatment.

Typically, a vector expressing the complement of the polynucleotides of the invention may be administered to a subject in need of such treatment. Antisense strategies may use a variety of approaches including the use of antisense oligonucleotides, injection of antisense RNA, ribozymes, DNazymes and transfection of antisense RNA expression vectors. Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken

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from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved using methods which are
5 well known in the art. (For example, see Goldman et al., 1997).

In a still further aspect of the invention there is provided the use of an isolated DNA molecule which is the complement of a DNA molecule of the invention and which
10 encodes an RNA molecule that hybridizes with the mRNA encoding a mutant sodium channel alpha subunit, in the manufacture of a medicament for the treatment of epilepsy, in particular generalised epilepsy with febrile seizures plus, as well as other disorders associated with sodium
15 channel dysfunction, including but not restricted to, malignant hyperthermia, myasthenia, episodic ataxia, neuropathic and inflammatory pain, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, myotonias such as hypo- and hyperkalaemic periodic
20 paralysis, paramyotonia congenita and potassium aggravated myotonia as well as cardiac arrhythmias such as long QT syndrome.

In a further aspect, a suitable agonist or modulator may include a small molecule that can restore wild-type
25 activity of the sodium channel containing mutations in the alpha subunit as described above, or may include an antibody to a mutant sodium channel that is able to restore channel function to a normal level.

Small molecules suitable for therapeutic applications
30 may be identified using nucleic acids and peptides of the invention in drug screening applications as described below.

In further embodiments, any of the agonists, antagonists, modulators, antibodies, complementary
35 sequences or vectors of the invention may be administered alone or in combination with other appropriate therapeutic agents. Selection of the appropriate agents may be made by

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those skilled in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using
5 this approach, therapeutic efficacy with lower dosages of each agent may be possible, thus reducing the potential for adverse side effects.

Drug screening

According to still another aspect of the invention,
10 peptides of the invention, particularly purified mutant sodium channel alpha subunit polypeptide and cells expressing these, are useful for the screening of candidate pharmaceutical agents in a variety of techniques. It will be appreciated that therapeutic agents
15 useful in the treatment of epilepsy, in particular generalised epilepsy with febrile seizures plus, as well as other disorders associated with sodium channel dysfunction, including but not restricted to, malignant hyperthermia, myasthenia, episodic ataxia, neuropathic and
20 inflammatory pain, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, myotonias such as hypo- and hyperkalaemic periodic paralysis, paramyotonia congenita and potassium aggravated myotonia as well as cardiac arrhythmias such as long QT syndrome, are likely
25 to show binding affinity to the polypeptides of the invention.

Such techniques include, but are not limited to, utilising eukaryotic or prokaryotic host cells that are stably transformed with recombinant molecules expressing
30 the polypeptide or fragment, preferably in competitive binding assays. Binding assays will measure the formation of complexes between a mutated sodium channel alpha subunit polypeptide or fragment and the agent being tested, or will measure the degree to which an agent being
35 tested will interfere with the formation of a complex between a mutated sodium channel alpha subunit polypeptide or fragment and a known ligand.

Another technique for drug screening provides high-throughput screening for compounds having suitable binding affinity to the mutant sodium channel alpha subunit polypeptides or sodium channels containing these (see PCT published application WO84/03564). In this stated technique, large numbers of small peptide test compounds can be synthesised on a solid substrate and can be assayed through mutant sodium channel or mutant sodium channel alpha subunit polypeptide binding and washing. Bound mutant sodium channel or mutant sodium channel alpha subunit polypeptide is then detected by methods well known in the art. In a variation of this technique, purified polypeptides of the invention can be coated directly onto plates to identify interacting test compounds.

The invention also contemplates the use of competition drug screening assays in which neutralizing antibodies capable of specifically binding the mutant sodium channel compete with a test compound for binding thereto. In this manner, the antibodies can be used to detect the presence of any peptide that shares one or more antigenic determinants of the mutant sodium channel.

The invention is particularly useful for screening compounds by using the polypeptides of the invention in transformed cells, transfected or injected oocytes, or animal models bearing mutated sodium channel alpha subunits (particularly those of the invention) such as transgenic animals or gene targeted (knock-in) animals (see below). A particular drug is added to the cells in culture or administered to an animal model containing a mutant sodium channel alpha subunit and the effect on the current of the channel is compared to the current of a cell or animal containing the wild-type sodium channel. Drug candidates that alter the current to a more normal level are useful for treating or preventing epilepsy, in particular generalised epilepsy with febrile seizures plus as well as other disorders associated with sodium channel dysfunction, as described above.

The polypeptides of the present invention may also be used for screening compounds developed as a result of combinatorial library technology. This provides a way to test a large number of different substances for their ability to modulate activity of a polypeptide. The use of peptide libraries is preferred (see WO 97/02048) with such libraries and their use known in the art.

A substance identified as a modulator of polypeptide function may be peptide or non-peptide in nature. Non-peptide "small molecules" are often preferred for many in vivo pharmaceutical applications. In addition, a mimic or mimetic of the substance may be designed for pharmaceutical use. The design of mimetics based on a known pharmaceutically active compound ("lead" compound) is a common approach to the development of novel pharmaceuticals. This is often desirable where the original active compound is difficult or expensive to synthesise or where it provides an unsuitable method of administration. In the design of a mimetic, particular parts of the original active compound that are important in determining the target property are identified. These parts or residues constituting the active region of the compound are known as its pharmacophore. Once found, the pharmacophore structure is modelled according to its physical properties using data from a range of sources including x-ray diffraction data and NMR. A template molecule is then selected onto which chemical groups which mimic the pharmacophore can be added. The selection can be made such that the mimetic is easy to synthesise, is likely to be pharmacologically acceptable, does not degrade in vivo and retains the biological activity of the lead compound. Further optimisation or modification can be carried out to select one or more final mimetics useful for in vivo or clinical testing.

It is also possible to isolate a target-specific antibody and then solve its crystal structure. In principle, this approach yields a pharmacophore upon which

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subsequent drug design can be based as described above. It may be possible to avoid protein crystallography altogether by generating anti-idiotypic antibodies (anti-ids) to a functional, pharmacologically active antibody.

5 As a mirror image of a mirror image, the binding site of the anti-ids would be expected to be an analogue of the original receptor. The anti-id could then be used to isolate peptides from chemically or biologically produced peptide banks.

10 Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

15 Diagnostic applications

Polynucleotide sequences of the invention may be used for the diagnosis of epilepsy, in particular generalised epilepsy with febrile seizures plus, as well as other disorders associated with sodium channel dysfunction, including but not restricted to, malignant hyperthermia, myasthenia, episodic ataxia, neuropathic and inflammatory pain, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia, myotonias such as hypo- and hyperkalaemic periodic paralysis, paramyotonia congenita, and potassium aggravated myotonia as well as cardiac arrhythmias such as long QT syndrome, and the use of the DNA molecules of the invention in diagnosis of these disorders, is therefore contemplated.

In another embodiment of the invention, the polynucleotides that may be used for diagnostic purposes include oligonucleotide sequences, genomic DNA and complementary RNA and DNA molecules. The polynucleotides may be used to detect and quantitate gene expression in biological samples. Genomic DNA used for the diagnosis may be obtained from body cells, such as those present in the blood, tissue biopsy, surgical specimen, or autopsy material. The DNA may be isolated and used directly for

detection of a specific sequence or may be amplified by the polymerase chain reaction (PCR) prior to analysis. Similarly, RNA or cDNA may also be used, with or without PCR amplification. To detect a specific nucleic acid sequence, hybridisation using specific oligonucleotides, restriction enzyme digest and mapping, PCR mapping, RNase protection, and various other methods may be employed. For instance direct nucleotide sequencing of amplification products from the sodium channel subunits can be employed. Sequence of the sample amplicon is compared to that of the wild-type amplicon to determine the presence (or absence) of nucleotide differences.

According to a further aspect of the invention there is provided the use of a polypeptide as described above in the diagnosis of epilepsy, in particular generalised epilepsy with febrile seizures plus, as well as other disorders associated with sodium channel dysfunction, as described above.

When a diagnostic assay is to be based upon mutant proteins constituting a sodium channel, a variety of approaches are possible. For example, diagnosis can be achieved by monitoring differences in the electrophoretic mobility of normal and mutant alpha subunit proteins that form part of the sodium channel. Such an approach will be particularly useful in identifying mutants in which charge substitutions are present, or in which insertions, deletions or substitutions have resulted in a significant change in the electrophoretic migration of the resultant protein. Alternatively, diagnosis may be based upon differences in the proteolytic cleavage patterns of normal and mutant proteins, differences in molar ratios of the various amino acid residues, or by functional assays demonstrating altered function of the gene products.

In another aspect, antibodies that specifically bind mutant sodium channels may be used for the diagnosis of epilepsy, or in assays to monitor patients being treated with agonists, antagonists, modulators or inhibitors of

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the mutant sodium channel. Antibodies useful for diagnostic purposes may be prepared in the same manner as described above for therapeutics. Diagnostic assays to detect mutant sodium channels include methods that utilize the antibody and a label to detect a mutant sodium channel in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labelled by covalent or non-covalent attachment of a reporter molecule.

A variety of protocols for measuring the presence of mutant sodium channels, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing epilepsy, in particular generalised epilepsy with febrile seizures plus, as well as other disorders associated with sodium channel dysfunction, as described above. The expression of a mutant channel is established by combining body fluids or cell extracts taken from test mammalian subjects, preferably human, with antibody to the channel under conditions suitable for complex formation. The amount of complex formation may be quantitated by various methods, preferably by photometric means. Antibodies specific for the mutant channel will only bind to individuals expressing the said mutant channel and not to individuals expressing only wild-type channels (ie normal individuals). This establishes the basis for diagnosing the disease.

Once an individual has been diagnosed with the disorder, effective treatments can be initiated. These may include administering a selective modulator of the mutant channel or an antagonist to the mutant channel such as an antibody or mutant complement as described above. Alternative treatments include the administering of a selective agonist or modulator to the mutant channel so as to restore channel function to a normal level.

35

Microarray

In further embodiments, complete cDNAs,

oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used as probes in a microarray. The microarray can be used to monitor the expression level of large numbers of genes simultaneously and to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, and to develop and monitor the activities of therapeutic agents. Microarrays may be prepared, used, and analyzed using methods known in the art. (For example, see Schena et al., 1996; Heller et al., 1997).

According to a further aspect of the present invention, neurological material obtained from animal models generated as a result of the identification of specific sodium channel alpha subunit human mutations, particularly those disclosed in the present invention, can be used in microarray experiments. These experiments can be conducted to identify the level of expression of specific sodium channel alpha subunits, or any cDNA clones from whole-brain libraries, in epileptic brain tissue as opposed to normal control brain tissue. Variations in the expression level of genes, including sodium channel alpha subunits, between the two tissues indicates their involvement in the epileptic process either as a cause or consequence of the original sodium channel mutation present in the animal model. Microarrays may be prepared, as described above.

30 Transformed hosts

The present invention also provides for the production of genetically modified (knock-out, knock-in and transgenic), non-human animal models transformed with the DNA molecules of the invention. These animals are useful for the study of the function of a sodium channel, to study the mechanisms of disease as related to a sodium channel, for the screening of candidate pharmaceutical

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compounds, for the creation of explanted mammalian cell cultures which express a mutant sodium channel and for the evaluation of potential therapeutic interventions.

Animal species which are suitable for use in the
5 animal models of the present invention include, but are not limited to, rats, mice, hamsters, guinea pigs, rabbits, dogs, cats, goats, sheep, pigs, and non-human primates such as monkeys and chimpanzees. For initial studies, genetically modified mice and rats are highly
10 desirable due to their relative ease of maintenance and shorter life spans. For certain studies, transgenic yeast or invertebrates may be suitable and preferred because they allow for rapid screening and provide for much easier handling. For longer term studies, non-human primates may
15 be desired due to their similarity with humans.

To create an animal model for a mutated sodium channel several methods can be employed. These include but are not limited to generation of a specific mutation in a homologous animal gene, insertion of a wild type human
20 gene and/or a humanized animal gene by homologous recombination, insertion of a mutant (single or multiple) human gene as genomic or minigene cDNA constructs using wild type or mutant or artificial promoter elements or insertion of artificially modified fragments of the
25 endogenous gene by homologous recombination. The modifications include insertion of mutant stop codons, the deletion of DNA sequences, or the inclusion of recombination elements (lox p sites) recognized by enzymes such as Cre recombinase.

30 To create a transgenic or gene targeted (knock-in) mouse, which are preferred, a mutant version of a sodium channel alpha subunit can be inserted into a mouse germ line using standard techniques of oocyte microinjection, or transfected into embryonic stem cells, respectively.
35 Alternatively, if it is desired to inactivate or replace an endogenous sodium channel alpha subunit gene, homologous recombination using embryonic stem cells may be

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applied.

For oocyte injection, one or more copies of the mutant sodium channel alpha subunit gene can be inserted into the pronucleus of a just-fertilized mouse oocyte. This oocyte is then reimplanted into a pseudo-pregnant foster mother. The liveborn mice can then be screened for integrants using analysis of tail DNA or DNA from other tissues for the presence of the particular human subunit gene sequence. The transgene can be either a complete genomic sequence injected as a YAC, BAC, PAC or other chromosome DNA fragment, a complete cDNA with either the natural promoter or a heterologous promoter, or a minigene containing all of the coding region and other elements found to be necessary for optimum expression.

According to still another aspect of the invention there is provided the use of genetically modified non-human animals as described above for the screening of candidate pharmaceutical compounds.

It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art, in Australia or in any other country.

Throughout this specification and the claims, the words "comprise", "comprises" and "comprising" are used in a non-exclusive sense, except where the context requires otherwise.

Brief Description of the Drawings

Preferred forms of the invention are described, by way of example only, with reference to the following examples and the accompanying drawings, in which:

Figure 1. Generalised epilepsy with febrile seizures plus (GEFS+) pedigrees are shown for the three families. DNA was not available from those individuals not assigned a letter (X, Y, or Z) or a 0. A: Pedigree of an Australian family with individual numbering for this

family based on Figure 1 in Scheffer & Berkovic (1997).
B: Pedigree of an Ashkenazi family. C: Pedigree of a
Druze family.

Figure 2. Schematic of the alpha subunit of the
sodium channel (SCN1A), showing the position of the three
mutations identified in this study.

Figure 3. Sodium channel amino acid alignments.
Alignment of sodium channel amino acids surrounding the
three SCN1A mutations.

10

Modes for Performing the Invention

Example 1: Clinical diagnosis of affected family members

A group of 53 unrelated probands with GEFS+
phenotypes were studied. These subjects were ascertained
on the basis of twin and family studies and on the basis
of routine clinical practice. Phenotypes in probands and
family members were classified as described elsewhere
(Scheffer & Berkovic 1997; Singh et al 1999). Familial
cases (n=36) were those in which at least one first-degree
relative of the proband had a phenotype within the GEFS+
spectrum. Informed consent was obtained from all subjects.

The Australian family in Figure 1A, which has been
described extensively elsewhere (Scheffer & Berkovic,
1997; Lopes-Cendes et al, 2000), is the original pedigree
leading to the initial delineation and description of the
GEFS+ syndrome.

The Israeli family in Figure 1B is of Ashkenazi
origin and spans six generations. Twelve family members
had seizures. In the two oldest members (I-2, III-3)
seizures had occurred in childhood but the data were
insufficient to allow classification of the phenotype. Of
the 10 other family members who had seizures, 3 had
febrile seizures with onset at age 9-13 months. All
attacks occurred with fever and offset occurred between 1
and 4 years with 1 to 7 attacks each. Five had febrile
seizures plus with onset at age 9-24 months, offset
between 5 and 41 years and 2 to 15 attacks each. Seizures

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during childhood were a mixture of febrile seizures and afebrile tonic-clonic seizures, whereas the rarely occurring seizures during teenage and adult years were all afebrile. Subject V-16 had a more severe phenotype with approximately 20 febrile seizures at age 6 months to 5 years, 10 afebrile tonic-clonic seizures at age 5 to 15 years and occasional complex partial seizures associated with mild learning difficulties. She was classified as having febrile seizures plus and complex partial seizures. Her older sister (V-15) had typical febrile seizures plus, but their younger brother (V-17), aged 14 years, had no febrile seizures but had two afebrile tonic-clonic seizures at ages 12 years 6 months and 14 years. For purposes of linkage analysis, he was regarded as affected, although he had only afebrile tonic-clonic seizures. All affected subjects were of normal or superior intellect, except V-16 (see above) and all had a normal neurological examination. Electroencephalography (EEG) studies had been performed infrequently during the active phase of the epilepsy, and the results usually either were normal or were reported to show generalised discharges.

The second Israeli family was of Druze origin; the parents were from different but proximate villages and were not known to be related. This family spans two generations, and four family members had seizures (Figure 1C). The proband aged 41 years (I-2) had had hundreds of tonic-clonic seizures, sometimes with fever. These began at age 4 years and continued, at a rate of approximately one per month, until the time of the study. The proband was mildly intellectually impaired. EEG showed generalized irregular spike-wave and polyspike-wave discharges, and febrile seizures plus was diagnosed. Of her four children, the oldest was unaffected (II-1), two had febrile seizures (II-2, II-4) and one had febrile seizures plus (II-3).

35

Example 2: Isolation and sequencing of SCN1A genomic clones

At the commencement of this study the full-length sequence of the human SCN1A gene was not known. To determine this sequence a human BAC library obtained from Genome Systems was initially screened to identify human
5 genomic sequence clones containing the SCN1A gene. The BAC filters were screened with a PCR product amplified with the primer pair 5' AGATGACCAGAGTGAATATGTGACTAC 3' (SEQ ID NO:13) and 5' CCAATGGTAAAATAATAATGGCGT 3' (SEQ ID NO:14) designed from the partial cDNA sequence of human SCN1A
10 (Genbank Accession Number X65362).

The BAC filters were hybridised and washed according to manufacturers recommendations. Initially, membranes were individually pre-hybridised in large glass bottles for at least 2 hours in 20 ml of 6X SSC; 0.5% SDS; 5X
15 Denhardt's; 100 ug/ml denatured salmon sperm DNA at 65°C. Overnight hybridisations with [α -³²P]dCTP labelled probes were performed at 65°C in 20 ml of a solution containing 6X SSC; 0.5% SDS; 100 ug/ml denatured salmon sperm DNA. Filters were washed sequentially in solutions of 2X SSC;
20 0.5% SDS (room temperature 5 minutes), 2X SSC; 0.1% SDS (room temperature 15 minutes) and 0.1X SSC; 0.5% SDS (37°C 1 hour if needed).

A number of BAC clones were identified from this hybridisation and BAC129e04 was selected for subcloning
25 and sequencing. DNA from this BAC clone was sheared by nebulisation (10psi for 45 seconds). Sheared DNA was then blunt ended using standard methodologies (Sambrook et al., 1989) and run on an agarose gel in order to isolate DNA in the 2-4 Kb size range. These fragments were cleaned from
30 the agarose using QIAquick columns (Qiagen), ligated into puc18 and used to transform competent XL-1 Blue *E. coli* cells. DNA was isolated from transformed clones and was sequenced using vector specific primers on an ABI377 sequencer to generate 1X coverage of the BAC clone.
35 Sequence data were assembled in contigs using the Phred, Phrap and Gap4 high throughput sequencing software. Exon-intron boundaries were predicted based on the rat *Scn1a*

cdNA sequence (Genbank Accession Number M22253) due to the full length human cdNA sequence of SCN1A not being known.

The human SCN1A gene was determined to be 8,381 base pair in length and is organised into 27 exons spanning over 100 Kb of genomic DNA. To facilitate a comparison with related sodium channels SCN4A, SCN5A and SCN8A, the first untranslated exon of SCN1A is designated exon 1A and the second exon, containing the start codon, remains exon 1 (Table 1). The SCN1A gene shows high homology to SCN2A and SCN3A at both the DNA and protein level. The close proximity of these genes to each other on chromosome 2 indicates likely duplication events during the evolution of the sodium channel gene family. Compared to SCN4A and SCN8A, additional sequence is present in the 3'UTR of SCN1A, giving the final exon an overall length of ~3.3 Kb.

Inspection of the splice junctions of SCN1A shows that there is close agreement with consensus splice motifs, with all introns bounded by GT-AG, except for two (introns 2 and 23). These introns exhibit deviation from the consensus splice pattern and are bounded by AT-AC terminal dinucleotides. These rare splice site variations are conserved in other characterised sodium channel subunits (SCN4A, SCN8A and the more distantly related SCN5A), indicating their ancient origin.

The intron positions are also highly conserved between sodium channel subunits, with most variation seen in the region that codes for the cytoplasmic loop between domains I and II of the gene (Table 1). Within this region, alternative splicing of exon 11 of SCN1A was found that was comparable to the alternative splicing of exon 10B in SCN8A (Plummer et al. 1998). Cytoplasmic loop 1 varies in both length and composition and is the proposed site of functional diversity among different sodium channels (Plummer & Meisler, 1999).

35

Example 3: Analysis of SCN1A for mutations in epilepsy

The determination of the genomic structure of SCN1A

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allowed the design of intronic primers (Table 2 and SEQ ID Numbers:15-88) to amplify each of the 27 exons of SCN1A in order to test for mutations in patients with generalised epilepsy with febrile seizures plus (GEFS+). A total of 53
5 unrelated patients (as described above) were screened by fluorescent single stranded conformation polymorphism (SSCP) analysis.

HEX-labelled primers were designed to amplify all exons of SCN1A (Table 2). A 30 ng sample of patient DNA
10 was amplified in a total volume of 10 ul. Products were separated on non-denaturing 4% polyacrylamide gels containing 2% glycerol using the GelScan 2000 (Corbett Research). PCR products showing a conformational change were reamplified from 100 ng of genomic DNA with
15 unlabelled primers and sequenced using the BigDye Terminator ready reaction kit (Perkin Elmer) according to manufacturers instructions.

A total of 53 unrelated patients with GEFS+ were screened by fluorescent SSCP, including two families
20 consistent with mapping to the same location as SCN1A on chromosome 2 (Figures 1A and 1B). No mutations were found in 17 sporadic cases of GEFS+ that were tested. Of the 36 families tested, 3 were found to have point mutations in SCN1A, which alter the amino acid sequence and are not
25 present in the control population (n=60). The phenotype in the family in Figure 1A previously had been mapped to chromosome 2 (Lopes-Cendes et al. 2000) and carries an A to T mutation at position 563 of the SCN1A coding sequence. This mutation segregates with affected family
30 members. This mutation in exon 4 of SCN1A results in a D188V amino acid substitution that lies just outside the S3 segment of domain I (Figure 2). The aspartic acid residue is conserved in all identified sodium channels in humans as well as in many different animal species, except
35 the jellyfish which has an arginine at this residue and the flatworm which has a serine (Figure 3). The published rat Scn2a sequence (Genbank Accession Number NM_012647)

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also has an arginine in place of the aspartic acid at residue 188.

5 A mutation in exon 21 (G to C nucleotide change at position 4057 of the SCN1A coding sequence) was found to segregate with GEFS+ in the Ashkenazi family (Figure 1B). This mutation changes a highly conserved amino acid (V1353L) located in the S5 segment of domain III (Figure 2). One family member (V-13) did not carry the mutation (Figure 1B). This was determined by testing the DNA of a
10 parent of this family member, since the subjects DNA was unavailable. This individual, who had typical febrile seizures that terminated at an early age, is likely to be a phenocopy. Mutations in the S5 segment of SCN4A that cause hyperkalemic periodic paralysis have been shown also
15 to affect the rate of channel inactivation (Bendahhou et al., 1999)

A third mutation (C to G nucleotide change at position 4968 of the SCN1A coding sequence) discovered in the Druze family (Figure 1C), changes an amino acid
20 (I1656M) in the S4 segment of domain IV (Figure 2). The S4 segment has a role in channel gating and mutations in this region of SCN1A reduce the rate of inactivation (Kuhn and Greef, 1996).

During the mutation screen of SCN1A several single
25 nucleotide polymorphisms (SNPs) were identified (Table 3). The R1928G variant was found at low frequency in both GEFS+ and control populations. The T1067A variant was common in both populations and the remaining SNPs identified did not alter the amino acid sequence of SCN1A
30 (Table 3).

Example 4: Analysis of a mutated sodium channels and sodium channel alpha subunits

The following methods are used to determine the
35 structure and function of mutated sodium channel or sodium channel alpha subunits.

Molecular biological studies

The ability of the mutated sodium channel as a whole or through individual alpha subunits to bind known and unknown proteins can be examined. Procedures such as the yeast two-hybrid system are used to discover and identify any functional partners. The principle behind the yeast two-hybrid procedure is that many eukaryotic transcriptional activators, including those in yeast, consist of two discrete modular domains. The first is a DNA-binding domain that binds to a specific promoter sequence and the second is an activation domain that directs the RNA polymerase II complex to transcribe the gene downstream of the DNA binding site. Both domains are required for transcriptional activation as neither domain can activate transcription on its own. In the yeast two-hybrid procedure, the gene of interest or parts thereof (BAIT), is cloned in such a way that it is expressed as a fusion to a peptide that has a DNA binding domain. A second gene, or number of genes, such as those from a cDNA library (TARGET), is cloned so that it is expressed as a fusion to an activation domain. Interaction of the protein of interest with its binding partner brings the DNA-binding peptide together with the activation domain and initiates transcription of the reporter genes. The first reporter gene will select for yeast cells that contain interacting proteins (this reporter is usually a nutritional gene required for growth on selective media). The second reporter is used for confirmation and while being expressed in response to interacting proteins it is usually not required for growth.

The nature of the genes and proteins interacting with the mutant sodium channels can also be studied such that these partners can also be targets for drug discovery.

Structural studies

Recombinant proteins corresponding to mutated sodium channel alpha subunits can be produced in bacterial,

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yeast, insect and/or mammalian cells and used in crystallographical and NMR studies. Together with molecular modeling of the protein, structure-driven drug design can be facilitated.

5

Example 5: Generation of polyclonal antibodies against a mutant sodium channel or sodium channel alpha subunit

Following the identification of new mutations in the alpha subunit of the sodium channel in individuals with
10 generalised epilepsy with febrile seizures plus, antibodies can be made to the mutant channel which can selectively bind and distinguish mutant from normal protein. Antibodies specific for mutagenised epitopes are especially useful in cell culture assays to screen for
15 cells which have been treated with pharmaceutical agents to evaluate the therapeutic potential of the agent.

To prepare polyclonal antibodies, short peptides can be designed homologous to a sodium channel subunit amino acid sequence. Such peptides are typically 10 to 15 amino
20 acids in length. These peptides should be designed in regions of least homology to other receptor subunits and should also have poor homology to the mouse orthologue to avoid cross species interactions in further down-stream experiments such as monoclonal antibody production.
25 Synthetic peptides can then be conjugated to biotin (Sulfo-NHS-LC Biotin) using standard protocols supplied with commercially available kits such as the PIERCE™ kit (PIERCE). Biotinylated peptides are subsequently complexed with avidin in solution and for each peptide complex, 2
30 rabbits are immunized with 4 doses of antigen (200 ug per dose) in intervals of three weeks between doses. The initial dose is mixed with Freund's Complete adjuvant while subsequent doses are combined with Freund's Immuno-adjuvant. After completion of the immunization, rabbits
35 are test bled and reactivity of sera is assayed by dot blot with serial dilutions of the original peptides. If rabbits show significant reactivity compared with pre-

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immune sera, they are then sacrificed and the blood collected such that immune sera can be separated for further experiments.

5 This procedure is repeated to generate antibodies against wild-type forms of receptor subunits. The antibodies specific for mutant sodium channels can subsequently be used to detect the presence and the relative level of the mutant forms in various tissues.

10 Example 6: Generation of monoclonal antibodies against a mutant sodium channel or sodium channel alpha subunit

Monoclonal antibodies can be prepared in the following manner. Immunogen, comprising intact mutated sodium channel or sodium channel alpha subunit peptides, 15 is injected in Freund's adjuvant into mice with each mouse receiving four injections of 10 ug to 100 ug of immunogen. After the fourth injection blood samples taken from the mice are examined for the presence of antibody to the immunogen. Immune mice are sacrificed, their spleens 20 removed and single cell suspensions are prepared (Harlow and Lane, 1988). The spleen cells serve as a source of lymphocytes, which are then fused with a permanently growing myeloma partner cell (Kohler and Milstein, 1975). Cells are plated at a density of 2×10^5 cells/well in 96 25 well plates and individual wells are examined for growth. These wells are then tested for the presence of sodium channel specific antibodies by ELISA or RIA using wild type or mutant subunit target protein. Cells in positive wells are expanded and subcloned to establish and confirm 30 monoclonality. Clones with the desired specificity are expanded and grown as ascites in mice followed by purification using affinity chromatography using Protein A Sepharose, ion-exchange chromatography or variations and combinations of these techniques.

35

Industrial Applicability

The present invention allows for the diagnosis and

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treatment of epilepsy or other disorders associated with sodium channel dysfunction, including but not restricted to, malignant hyperthermia, myasthenia, episodic ataxia, neuropathic and inflammatory pain, Alzheimer's disease, 5 Parkinson's disease, schizophrenia, hyperekplexia, myotonias such as hypo- and hyperkalaemic periodic paralysis, paramyotonia congenita and potassium aggravated myotonia as well as cardiac arrhythmias such as long QT syndrome. In particular, the present invention allows for 10 the diagnosis and treatment of generalised epilepsy with febrile seizures plus.

TABLE 1

Comparison of Exon Sizes of SCN1A with Other Human SCNA
5 Subunits

SCN1A		SCN4A		SCN8A		SCN5A	
Exon No.	Exon Size	Exon No.	Exon Size	Exon No.	Exon Size	Exon No.	Exon Size
1A	217	-	-	-	-	1	98
1	313	1	661	1	276	2	324
2	119	2	119	2	121	3	119
3	90	3	90	3	88	4	90
4	129	4	129	4	129	5	129
5	D I	5	92	5	92	6	92
6		6	333	6	222	7	231
7		7	64	7	64	8	64
8		8	142	8	142	9	142
9		9	210	9	207	10	198
10		10A	154	10A	294	11	180
11	C loop1	10B	-	10B	396	12	372
12		10C	-	10C	133	13	133
13		11	239	11	239	14	239
14	D II	12	174	12	174	15	174
15		13	357	13	357	16	351
16		14	477	14	471	17	441
	C loop2					18	162
17		15	136	15	118	19	121
18		16	155	16	155	20	155
19		17	174	17	174	21	174
20	D III	18A	123	18A	123	22	123
21		19	279	19	285	23	282
22		20	54	20	54	24	54
23		21	138	21	138	25	138
24		22	105	22	105	26	105
25	D IV	23	271	23	271	27	271
26		24	>2242	24	>1158	28	3257

Note: D: Transmembrane domain; C: Cytoplasmic loop.

TABLE 2**Primer Sequences Used for Mutation Analysis of SCN1A**

Exon	Forward Primer	Reverse Primer	Size (bp)
1A	TACCATAGAGTGAGGCGAGG	ATGGACTTCCTGCTCTGCC	356
1	CCTCTAGCTCATGTTTCATGAC	TGCAGTAGGCAATTAGCAGC	448
2	CTAATTAAGAAGAGATCCAGTGACAG	GCTATAAAGTGCTTACAGATCATGTAC	356
3	CCCTGAATTTTGGCTAAGCTGCAG	CTACATTAAGACACAGTTTCAAAATCC	263
4	GGGCTACGTTTCATTGTATG	GCAACCTATTCTTAAAGCATAAGACTG	355
5	AGGCTCTTTGTACCTACAGC	CATGTAGGGTCCGTCTCATT	199
6	CACACGTGTTAAGTCTTCATAGT	AGCCCCCTCAAGTATTTATCCT	394
7	GAACCTGACCTTCCTGTCTC	GTTGGCTGTTATCTTCAGTTTC	241
8	GACTAGGCAATATCATAGCATAG	CTTCTACTATATTATCATCCGG	320
9	TTGAAAGTTGAAGCCACCAC	CCACCTGCTCTTAGGTACTC	363
10	GCCATGCAAAATAC TTCAGCCC	CACAACAGTGGTTGATTTCAGTTG	480
11a	TGAATGCTGAAATCTCCTTCTAC	CTCAGGTTGCTGTTGCGTCTC	306
11b	GATAACGAGAGCCGTAGAGAT	TCTGTAGAAACACTGGCTGG	315
12	CATGAAATTCAGTGTGCACC	CAGCTCTTGAATTAGACTGTC	347
13a	ATCCTTGGGAGGTTTAGAGT	CATCACAACCAGGTTGACAAC	292
13b	CTGGGACTGTTCTCCATATTG	GCATGAAGGATGGTTGAAAG	277
14	CATTGTGGGAAAATAGCATAAGC	GCTATGCAGAACCCCTGATTG	338
15a	TGAGACGGTTAGGGCAGATC	AGAAGTCATTTCATGTGCCAGC	348
15b	CTGCAAGATCGCCAGTGATTG	ACATGTGACAATGTGCAGG	276
16a	GTGGTGTTCCTTCTCATCAAG	TCTGCTGTATGATTGGACATAC	387
16b	CAACAGTCCTTCATTAGGAAAC	ACCTTCCCACACCTATAGAATC	353
17	CTTGGCAGGCAACTTATTACC	CAAGCTGCACTCCAAATGAAAG	232
18	TGGAAGCAGAGACACTTTATCTAC	GTGCTGTATCACCTTTTCTTAATC	234
19	CCTATTCCAATGAAATGTCATATG	CAAGCTACCTTGAACAGAGAC	318
20	CTACACATTGAATGATGATTCTGT	GCTATATACAATACTTCAGGTTCT	216
21a	ACCAGAGATTACTAGGGGAAT	CCATCGAGCAGTCTCATTTCT	303
21b	ACAACTGGTGACAGGTTTGAC	CTGGGCTCATAAACTTGTACTAAC	297
22	ACTGTCTTGGTCCAAAATCTG	TTCGATTAAATTTTACCACCTGATC	267
23	AGCACCAGTGACATTTCACAC	GGCAGAGAAAACACTCCAAGG	272
24	GACACAGTTTTAACCAGTTTG	TGTGAGACAAGCATGCAAGTT	207
25	CAGGGCCAATGACTACTTTGC	CTGATTGCTGGGATGATCTTGAATC	477
26a	CGCATGATTTCTTCACTGGTTGG	GCGTAGATGAACATGACTAGG	247
26b	TCCTGCGTGTGTTAACATCGG	ATTCCAACAGATGGGTTCCCA	288
26c	TGGAAGCTCAGTTAAGGGAGA	AGCGCAGCTGCAAACTGAGAT	261
26d	CCGATGCAACTCAGTTTCATGGA	GTAGTGATTGGCTGATAGGAG	274
26e	AGAGCGATTTCATGGCTTCCAATCC	TGCCTTCTTGCTCATGTTTTCCACA	335
26f	CCTATGACCGGGTGACAAAGCC	TGCTGACAAGGGGTCACTGTCT	242

Note: Primer sequences are listed 5' to 3'. Due to the large size of exons 11, 13, 15, 16, 21 and 26, the exons were split into two or more overlapping amplicons.

5

TABLE 3

SCN1A Polymorphisms Identified				
SCN1A polymorphism			Frequency (%)	
Position	Mutation	Amino Acid Change	GEFS+	Normal
Intron 13	IVS13-37C>A	-	2.4	8.6
Exon 14	c.2522C>G	-	2.4	8.6
Intron 15	IVS15+54A>G	-	36.3	23.6
Exon 15	c.2889T>C	-	1.2	0.0
Exon 16	c.3199G>A	T1067A	29.5	30.8
Exon 26	c.5782C>G	R1928G	1.2	1.7

Note: Total GEFS+ samples = 53; Total normal samples=60.

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Claims

1. An isolated nucleic acid molecule encoding a mutant alpha subunit of a mammalian voltage-gated sodium channel, wherein a mutation event selected from the group consisting of point mutations, deletions, insertions and rearrangements has occurred and said mutation event disrupts the functioning of an assembled sodium channel so as to produce an epilepsy phenotype, with the proviso that the mutation event is not a C2624T transition or a G4943A transition in an alpha-1 subunit.
2. An isolated nucleic acid molecule as claimed in claim 1 wherein said mutation event occurs in the nucleotides encoding an intracellular loop.
3. An isolated nucleic acid molecule as claimed in claim 2 wherein said mutation event occurs in the nucleotides encoding the intracellular loop between transmembrane segments 2 and 3 of domain I.
4. An isolated nucleic acid molecule as claimed in claim 3 wherein said mutation event is a point mutation.
5. An isolated nucleic acid molecule as claimed in claim 4 wherein said mutation event results in replacement of an aspartic acid residue at amino acid position 188 of the alpha-1 subunit of a sodium channel.
6. An isolated nucleic acid molecule as claimed in claim 5 wherein the aspartic acid residue at amino acid position 188 of the alpha-1 subunit of a sodium channel is replaced by a valine.
7. An isolated nucleic acid molecule as claimed in claim 6 wherein said mutation event is an A to T nucleotide substitution at position 563 of the coding sequence of the alpha-1 subunit of a sodium channel.

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8. An isolated nucleic acid molecule as claimed in claim 7 comprising the nucleotide sequence set forth in SEQ ID NO:1.

5 9. An isolated nucleic acid molecule as claimed in claim 1 wherein said mutation event takes place in the nucleotides encoding an S5 segment of a transmembrane domain.

10 10. An isolated nucleic acid molecule as claimed in claim 9 wherein said mutation event occurs in the nucleotides encoding the S5 segment of domain III.

15 11. An isolated nucleic acid molecule as claimed in claim 10 wherein said mutation event is a point mutation.

20 12. An isolated nucleic acid molecule as claimed in claim 11 wherein said mutation event results in replacement of a valine residue at amino acid position 1353 of the alpha-1 subunit of a sodium channel.

25 13. An isolated nucleic acid molecule as claimed in claim 12 wherein the valine residue at amino acid position 1353 of the alpha-1 subunit of a sodium channel is replaced by a leucine.

30 14. An isolated nucleic acid molecule as claimed in claim 13 wherein said mutation event is a G to C nucleotide substitution at position 4057 of the coding sequence of the alpha-1 subunit of a sodium channel.

35 15. An isolated nucleic acid molecule as claimed in claim 14 comprising the nucleotide sequence set forth in SEQ ID NO:3.

16. An isolated nucleic acid molecule as claimed in claim 1 wherein said mutation event occurs in the nucleotides

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encoding an S4 segment of a transmembrane domain.

17. An isolated nucleic acid molecule as claimed in claim
16 wherein said mutation event occurs in the nucleotides
5 encoding the S4 segment of domain IV.

18. An isolated nucleic acid molecule as claimed in claim
17 wherein said mutation event is a point mutation.

10 19. An isolated nucleic acid molecule as claimed in claim
18 wherein said mutation event results in replacement of
an isoleucine residue at amino acid position 1656 of the
alpha-1 subunit of a sodium channel.

15 20. An isolated nucleic acid molecule as claimed in claim
19 wherein the isoleucine residue at amino acid position
1656 of the alpha-1 subunit of a sodium channel is
replaced by a methionine.

20 21. An isolated nucleic acid molecule as claimed in claim
20 wherein said mutation event is a C to G nucleotide
substitution at position 4968 of the coding sequence of
the alpha-1 subunit of a sodium channel.

25 22. An isolated nucleic acid molecule as claimed in claim
21 comprising the nucleotide sequence set forth in SEQ ID
NO:5.

23. An isolated nucleic acid molecule as claimed in any
30 one of claims 1 to 22 in which one or more additional
mutation events selected from the group consisting of
point mutations, deletions, insertions and rearrangements
have occurred.

35 24. An isolated nucleic acid molecule as claimed in claim
23 wherein said one or more additional mutation events are
point mutations which result in conservative amino acid

substitutions.

25. An isolated nucleic acid molecule encoding a mutant
alpha subunit of a mammalian voltage-gated sodium channel,
5 wherein a mutation event selected from the group
consisting of point mutations, deletions, insertions and
rearrangements has occurred in an intracellular loop, in
the S4 segment of domain IV at nucleotide position 4968 of
the alpha-1 subunit coding sequence or homologous
10 nucleotide position in the coding sequence of other alpha
subunits, or in an S5 segment of a transmembrane domain so
as to produce an epilepsy phenotype.

26. An isolated nucleic acid molecule consisting of the
15 nucleotide sequence set forth in SEQ ID NO:1.

27. An isolated nucleic acid molecule consisting of the
nucleotide sequence set forth in SEQ ID NO:3.

20 28. An isolated nucleic acid molecule consisting of the
nucleotide sequence set forth in SEQ ID NO:5.

29. An isolated nucleic acid molecule selected from the
group consisting of DNA molecules comprising the
25 nucleotide sequence set forth in any one of SEQ ID NO:7,
8, 9 ,11 and 89.

30. An isolated polypeptide, said polypeptide being a
mutant alpha subunit of a mammalian voltage-gated sodium
30 channel, wherein a mutation event selected from the group
consisting of substitutions, deletions, insertions and
rearrangements has occurred and said mutation event
disrupts the functioning of an assembled sodium channel so
as to produce an epilepsy phenotype, with the proviso that
35 the mutation event is not a T875M transition or a R1648H
transition in an alpha-1 subunit.

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31. An isolated polypeptide as claimed in claim 30 wherein said mutation event occurs in an intracellular loop.

5 32. An isolated polypeptide as claimed in claim 30 wherein said mutation event occurs in an intracellular loop between transmembrane segments 2 and 3 of domain I.

10 33. An isolated polypeptide as claimed in claim 30 wherein said mutation event is a substitution.

15 34. An isolated polypeptide as claimed in claim 33 wherein the substitution involves replacement of an aspartic acid residue at position 188 of the alpha-1 subunit of a sodium channel.

20 35. An isolated polypeptide as claimed in claim 34 wherein the aspartic acid residue is replaced with a valine residue.

36. An isolated polypeptide as claimed in claim 35 comprising the amino acid sequence set forth in SEQ ID NO:2.

25 37. An isolated polypeptide as claimed in claim 30 wherein the mutation event occurs in an S5 segment of a transmembrane domain.

30 38. An isolated polypeptide as claimed in claim 37 wherein said mutation event occurs in the S5 segment of domain III.

35 39. An isolated polypeptide as claimed in claim 38 wherein said mutation event is a substitution.

40. An isolated polypeptide as claimed in claim 39 wherein the substitution involves replacement of a valine

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residue at position 1353 of the alpha-1 subunit of a sodium channel.

41. An isolated polypeptide as claimed in claim 40
5 wherein the valine residue is replaced with a leucine residue.

42. An isolated polypeptide as claimed in claim 41
10 comprising the amino acid sequence set forth in SEQ ID NO:4.

43. An isolated polypeptide as claimed in claim 30
15 wherein said mutation event occurs in an S4 segment of a transmembrane domain.

44. An isolated polypeptide as claimed in claim 41
wherein said mutation event occurs in the S4 segment of domain IV.

20 45. An isolated polypeptide as claimed in claim 44 wherein an isoleucine residue at position 1656 of the alpha-1 subunit of a sodium channel is replaced.

25 46. An isolated polypeptide as claimed in claim 45 wherein the isoleucine residue is replaced with a methionine residue.

30 47. An isolated polypeptide as claimed in claim 46 comprising the amino acid sequence set forth in SEQ ID NO:6.

35 48. An isolated polypeptide, said polypeptide being a mutant α -subunit of a mammalian voltage-gated sodium channel, wherein a mutation event selected from the group of substitutions, deletions, insertions and rearrangements has occurred in an intracellular loop, in the S4 segment of domain IV at amino acid position 1656 of the alpha-1

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subunit or homologous amino acid position of other alpha subunits, or in an S5 segment of a transmembrane domain.

5 49. An isolated polypeptide having the amino acid sequence set forth in SEQ ID NO:2.

50. An isolated polypeptide having the amino acid sequence set forth in SEQ ID NO:4.

10 51. An isolated polypeptide having the amino acid sequence set forth in SEQ ID NO:6.

15 52. An isolated polypeptide, said polypeptide being an assembled mammalian voltage-gated sodium channel comprising an alpha subunit as defined in any one of claims 30 to 51.

20 53. An isolated polypeptide selected from the group consisting of polypeptides with the amino acid sequence set forth in SEQ ID NO:10 or SEQ ID NO:12.

54. A cell transformed with an isolated nucleic acid molecule as claimed in any one of claims 1 to 29.

25 55. A cell as claimed in claim 54 which is an eukaryotic cell or bacterial cell.

56. A method of preparing a polypeptide comprising the steps of:

- 30 (1) culturing cells as claimed in claim 54 or 55 under conditions effective for polypeptide production; and
(2) harvesting the polypeptide.

35 57. A polypeptide prepared by the method of claim 56.

58. An antibody which is immunologically reactive with a mutant polypeptide as defined in any one of claims 30 to

52, but not with a wild-type mammalian voltage-gated sodium channel.

59. An antibody as claimed in claim 58 which is selected from the group consisting of a monoclonal antibody, a humanised antibody, a chimaeric antibody or an antibody fragment including a Fab fragment, (Fab')₂ fragment, Fv fragment, single chain antibodies and single domain antibodies.

60. A method of treating disorders associated with sodium channel dysfunction, comprising administering a selective agonist, antagonist or modulator of the sodium channel when it has undergone a mutation event as defined in any one of claims 30 to 48 to a patient in need of such treatment.

61. The use of a selective agonist, antagonist or modulator of the sodium channel when it has undergone a mutation event as defined in any one of claims 30 to 48 in the manufacture of a medicament for the treatment of a disorder associated with sodium channel dysfunction.

62. A method of treating disorders associated with sodium channel dysfunction, comprising administering an isolated DNA molecule which is the complement (antisense) of a nucleic acid molecule as defined in any one of claims 1 to 29 and which encodes an RNA molecule that hybridizes with the mRNA encoding a mutant sodium channel alpha subunit, to a subject in need of such treatment.

63. The use of an isolated DNA molecule which is the complement of a nucleic acid molecule as defined in any one of claims 1 to 29 and which encodes an RNA molecule that hybridizes with the mRNA encoding a mutant sodium channel alpha subunit, in the manufacture of a medicament for the treatment of disorders associated with sodium channel dysfunction.

64. A method of treating disorders associated with sodium channel dysfunction comprising administration of an antibody as defined in claim 58 or 59.

5

65. Use of a polypeptide as claimed in any one of claims 30 to 53 or 57 for the screening of candidate pharmaceutical agents.

10 66. Use as claimed in claim 65 wherein high throughput screening techniques are employed.

15 67. A genetically modified non-human animal transformed with an isolated nucleic acid molecule as defined in any one of claims 1 to 29.

68. A genetically modified non-human animal as claimed in claim 67 in which the animal is selected from the group consisting of rats, mice, hamsters, guinea pigs, rabbits, 20 dogs, cats, goats, sheep, pigs and non-human primates such as monkeys and chimpanzees.

69. The use of a genetically modified non-human animal as claimed in claim 67 or 68 in the screening of candidate 25 pharmaceutical compounds.

70. The use of a cell as claimed in claim 54 to 55 in the screening of candidate pharmaceuticals.

30 71. An expression vector comprising a DNA molecule as claimed in any one of claims 1 to 29.

72. A microarray comprising a complete cDNA, an oligonucleotide or a longer fragment derived from any of 35 the polynucleotide sequences defined in claims 1 to 29.

73. The use of a DNA molecule as claimed in any one of

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claims 1 to 29 in the diagnosis of epilepsy, in particular generalised epilepsy with febrile seizures plus, and other disorders associated with sodium channel dysfunction.

5 74. The use of a polypeptide as defined in any one of claims 30 to 53 or 57 in the diagnosis of epilepsy, in particular generalised epilepsy with febrile seizures plus, as well as other disorders associated with sodium channel dysfunction.

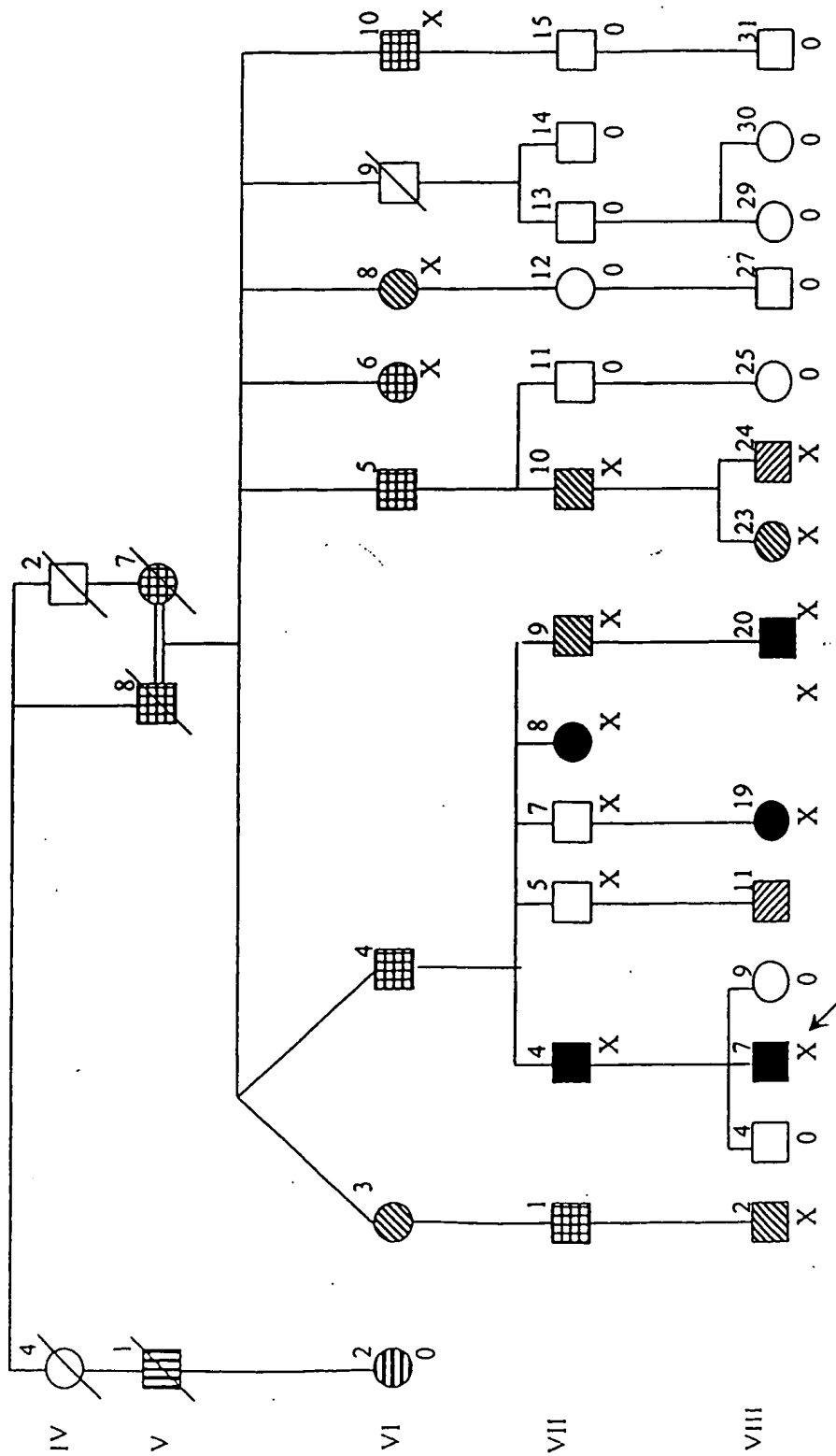
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75. The use of an antibody as defined in claims 58 or 59 in the diagnosis of epilepsy, in particular generalised epilepsy with febrile seizures plus, as well as other disorders associated with sodium channel dysfunction.

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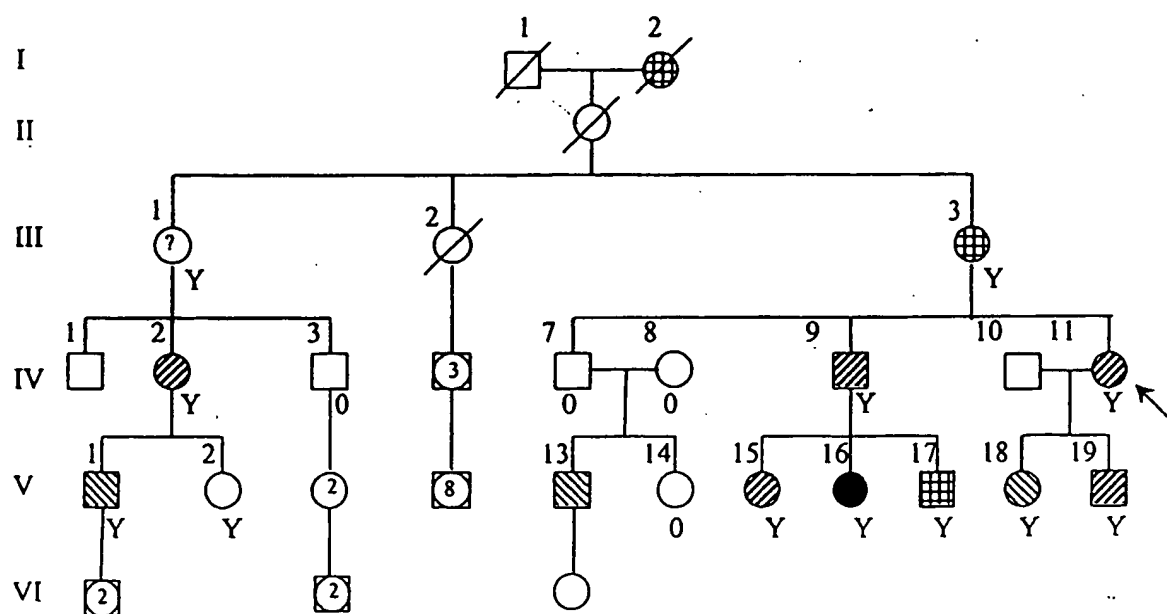
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Figure 1A



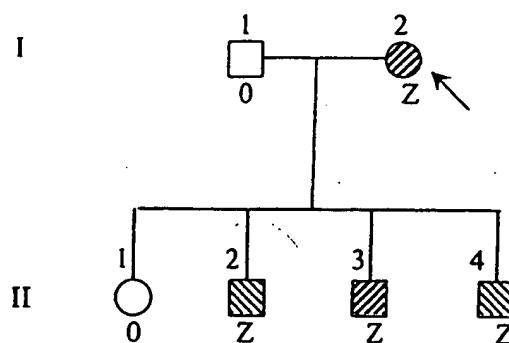
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Figure 1B



3/5

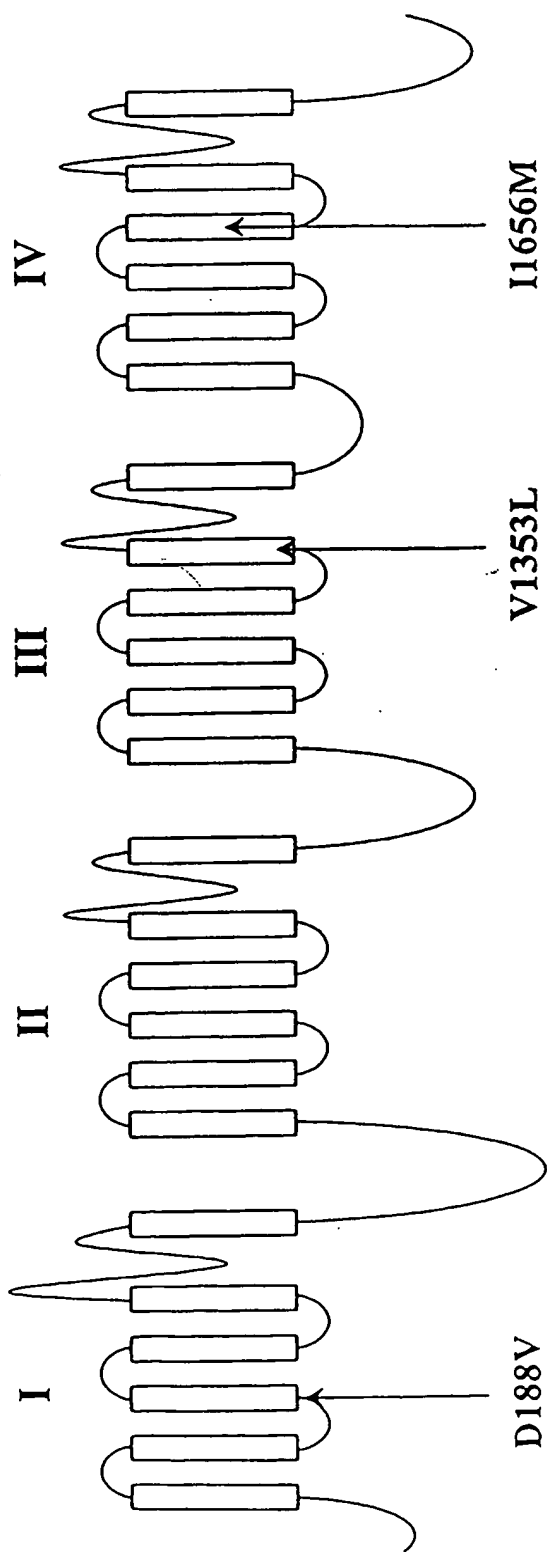
Figure 1C



	febrile seizures (FS)	X	D188V
	febrile seizures plus (FS+)	Y	V1353L
	FS+, extended phenotype	Z	I1656M
	Unclassified	0	no mutation
	Partial epilepsy		
	Juvenile myoclonic epilepsy		

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Figure 2



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Figure 3

i) D188V

	F	T	F	L	R	D	P	W	N	W	L
SCN1A	-	-	-	-	-	-	-	-	-	-	-
RAT SCN1A	-	-	-	-	-	-	-	-	-	-	-
SCN2A	-	-	-	-	-	-	-	-	-	-	-
SCN3A	-	-	-	-	-	-	-	-	-	-	-
SCN4A	-	-	-	-	-	-	-	-	-	-	-
SCN5A	-	-	-	-	-	-	-	-	-	-	-
SCN6A	-	S	-	-	G	-	-	-	-	-	-
SCN8A	-	-	-	-	-	-	-	-	-	-	-
SCN9A	-	-	-	-	-	-	-	-	-	-	-
SCN10A	-	-	Y	-	-	-	-	-	-	-	-
SCN11A	-	S	-	-	-	-	-	-	-	-	-
SCN12A	-	S	-	-	-	-	-	-	-	-	-
EL. EEL	-	-	-	-	-	-	-	-	-	-	-
DROS	-	-	Y	-	-	-	A	-	-	-	-
SQUID	-	-	Y	-	-	-	A	-	-	-	-
FLATWORM	-	-	Y	-	-	S	I	-	-	-	-
JELLYFISH	Y	S	Y	-	-	N	S	-	-	-	-

ii) V1353L

	M	N	V	L	L	V	C	L	I	F	W
SCN1A	-	-	-	-	-	-	-	-	-	-	-
RAT SCN1A	-	-	-	-	-	-	-	-	-	-	-
SCN2A	-	-	-	-	-	-	-	-	-	-	-
SCN3A	-	-	-	-	-	-	-	-	-	-	-
SCN4A	-	-	-	-	-	-	-	-	-	-	-
SCN5A	-	-	-	-	-	-	-	-	-	-	-
SCN6A	L	-	-	F	-	-	-	M	I	-	-
SCN8A	-	-	-	-	-	-	-	-	-	-	-
SCN9A	-	-	-	-	-	-	-	-	-	-	-
SCN10A	-	-	-	-	-	-	-	-	-	-	-
SCN11A	L	-	-	-	-	-	-	-	-	-	-
SCN12A	L	-	-	-	-	-	-	-	-	-	-
EL. EEL	-	-	-	-	-	-	-	-	-	-	-
DROS	F	-	-	-	-	-	-	-	-	-	-
SQUID	F	-	-	-	-	-	-	V	-	-	-
FLATWORM	F	-	-	M	V	-	-	V	-	-	-
JELLYFISH	A	-	-	-	-	-	G	V	-	-	-

iii) I1656M

	K	G	A	K	G	I	R	T	L	L	F
SCN1A	-	-	-	-	-	-	-	-	-	-	-
RAT SCN1A	-	-	-	-	-	-	-	-	-	-	-
SCN2A	-	-	-	-	-	-	-	-	-	-	-
SCN3A	-	-	-	-	-	-	-	-	-	-	-
SCN4A	R	-	-	-	-	-	-	-	-	-	-
SCN5A	R	-	-	-	-	-	-	-	-	-	-
SCN6A	-	-	P	-	V	F	H	N	-	M	L
SCN8A	-	-	-	-	-	-	-	-	-	-	-
SCN9A	-	-	-	-	-	-	-	-	-	-	-
SCN10A	R	A	-	-	-	-	-	-	-	-	-
SCN11A	R	A	-	-	-	-	-	-	-	-	-
SCN12A	R	A	-	-	-	-	-	-	-	-	-
EL. EEL	-	-	-	-	-	-	-	-	-	-	-
DROS	-	-	-	-	-	-	-	-	-	-	-
SQUID	-	S	-	-	-	-	-	-	-	-	-
FLATWORM	-	S	-	R	-	-	-	-	-	-	-
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SCN1APCT1.ST25.txt

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3240

SCN1APCT1.ST25.txt

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3300

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SCN1APCT1.ST25.txt

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SCN1APCT1.ST25.txt

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8280

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Phe	Thr	Arg	Glu	Ser	Leu	Ala	Ala	Ile	Glu	Arg	Arg	Ile	Ala	Glu	Glu
			20					25					30		

Lys	Ala	Lys	Asn	Pro	Lys	Pro	Asp	Lys	Lys	Asp	Asp	Asp	Glu	Asn	Gly
		35					40					45			

Pro	Lys	Pro	Asn	Ser	Asp	Leu	Glu	Ala	Gly	Lys	Asn	Leu	Pro	Phe	Ile
	50					55					60				

Tyr	Gly	Asp	Ile	Pro	Pro	Glu	Met	Val	Ser	Glu	Pro	Leu	Glu	Asp	Leu
65					70					75					80

Asp	Pro	Tyr	Tyr	Ile	Asn	Lys	Lys	Thr	Phe	Ile	Val	Leu	Asn	Lys	Leu
				85					90					95	

Lys	Ala	Ile	Phe	Arg	Phe	Ser	Ala	Thr	Ser	Ala	Leu	Tyr	Ile	Leu	Thr
			100					105					110		

Pro	Phe	Asn	Pro	Leu	Arg	Lys	Ile	Ala	Ile	Lys	Ile	Leu	Val	His	Ser
		115					120					125			

Leu	Phe	Ser	Met	Leu	Ile	Met	Cys	Thr	Ile	Leu	Thr	Asn	Cys	Val	Phe
	130					135					140				

Met	Thr	Met	Ser	Asn	Pro	Pro	Asp	Trp	Thr	Lys	Asn	Val	Glu	Tyr	Thr
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SCN1APCT1.ST25.txt

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Gly Phe Cys Leu Glu Asp Phe Thr Phe Leu Arg Val Pro Trp Asn Trp
 180 185 190

Leu Asp Phe Thr Val Ile Thr Phe Ala Tyr Val Thr Glu Phe Val Asp
 195 200 205

Leu Gly Asn Val Ser Ala Leu Arg Thr Phe Arg Val Leu Arg Ala Leu
 210 215 220

Lys Thr Ile Ser Val Ile Pro Gly Leu Lys Thr Ile Val Gly Ala Leu
 225 230 235 240

Ile Gln Ser Val Lys Lys Leu Ser Asp Val Met Ile Leu Thr Val Phe
 245 250 255

Cys Leu Ser Val Phe Ala Leu Ile Gly Leu Gln Leu Phe Met Gly Asn
 260 265 270

Leu Arg Asn Lys Cys Ile Gln Trp Pro Pro Thr Asn Ala Ser Leu Glu
 275 280 285

Glu His Ser Ile Glu Lys Asn Ile Thr Val Asn Tyr Asn Gly Thr Leu
 290 295 300

Ile Asn Glu Thr Val Phe Glu Phe Asp Trp Lys Ser Tyr Ile Gln Asp
 305 310 315 320

Ser Arg Tyr His Tyr Phe Leu Glu Gly Phe Leu Asp Ala Leu Leu Cys
 325 330 335

Gly Asn Ser Ser Asp Ala Gly Gln Cys Pro Glu Gly Tyr Met Cys Val
 340 345 350

Lys Ala Gly Arg Asn Pro Asn Tyr Gly Tyr Thr Ser Phe Asp Thr Phe
 355 360 365

Ser Trp Ala Phe Leu Ser Leu Phe Arg Leu Met Thr Gln Asp Phe Trp

SCN1APCT1.ST25.txt

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375

380

Glu Asn Leu Tyr Gln Leu Thr Leu Arg Ala Ala Gly Lys Thr Tyr Met
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Ile Phe Phe Val Leu Val Ile Phe Leu Gly Ser Phe Tyr Leu Ile Asn
 405 410 415

Leu Ile Leu Ala Val Val Ala Met Ala Tyr Glu Glu Gln Asn Gln Ala
 420 425 430

Thr Leu Glu Glu Ala Glu Gln Lys Glu Ala Glu Phe Gln Gln Met Ile
 435 440 445

Glu Gln Leu Lys Lys Gln Gln Glu Ala Ala Gln Gln Ala Ala Thr Ala
 450 455 460

Thr Ala Ser Glu His Ser Arg Glu Pro Ser Ala Ala Gly Arg Leu Ser
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Asp Ser Ser Ser Glu Ala Ser Lys Leu Ser Ser Lys Ser Ala Lys Glu
 485 490 495

Arg Arg Asn Arg Arg Lys Lys Arg Lys Gln Lys Glu Gln Ser Gly Gly
 500 505 510

Glu Glu Lys Asp Glu Asp Glu Phe Gln Lys Ser Glu Ser Glu Asp Ser
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Ile Arg Arg Lys Gly Phe Arg Phe Ser Ile Glu Gly Asn Arg Leu Thr
 530 535 540

Tyr Glu Lys Arg Tyr Ser Ser Pro His Gln Ser Leu Leu Ser Ile Arg
 545 550 555 560

Gly Ser Leu Phe Ser Pro Arg Arg Asn Ser Arg Thr Ser Leu Phe Ser
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Phe Arg Gly Arg Ala Lys Asp Val Gly Ser Glu Asn Asp Phe Ala Asp
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SCN1APCT1.ST25.txt

Asp Glu His Ser Thr Phe Glu Asp Asn Glu Ser Arg Arg Asp Ser Leu
 595 600 605

Phe Val Pro Arg Arg His Gly Glu Arg Arg Asn Ser Asn Leu Ser Gln
 610 615 620

Thr Ser Arg Ser Ser Arg Met Leu Ala Val Phe Pro Ala Asn Gly Lys
 625 630 635 640

Met His Ser Thr Val Asp Cys Asn Gly Val Val Ser Leu Val Gly Gly
 645 650 655

Pro Ser Val Pro Thr Ser Pro Val Gly Gln Leu Leu Pro Glu Val Ile
 660 665 670

Ile Asp Lys Pro Ala Thr Asp Asp Asn Gly Thr Thr Thr Glu Thr Glu
 675 680 685

Met Arg Lys Arg Arg Ser Ser Ser Phe His Val Ser Met Asp Phe Leu
 690 695 700

Glu Asp Pro Ser Gln Arg Gln Arg Ala Met Ser Ile Ala Ser Ile Leu
 705 710 715 720

Thr Asn Thr Val Glu Glu Leu Glu Glu Ser Arg Gln Lys Cys Pro Pro
 725 730 735

Cys Trp Tyr Lys Phe Ser Asn Ile Phe Leu Ile Trp Asp Cys Ser Pro
 740 745 750

Tyr Trp Leu Lys Val Lys His Val Val Asn Leu Val Val Met Asp Pro
 755 760 765

Phe Val Asp Leu Ala Ile Thr Ile Cys Ile Val Leu Asn Thr Leu Phe
 770 775 780

Met Ala Met Glu His Tyr Pro Met Thr Asp His Phe Asn Asn Val Leu
 785 790 795 800

Thr Val Gly Asn Leu Val Phe Thr Gly Ile Phe Thr Ala Glu Met Phe
 805 810 815

SCN1APCT1.ST25.txt

Leu Lys Ile Ile Ala Met Asp Pro Tyr Tyr Tyr Phe Gln Glu Gly Trp
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Asn Ile Phe Asp Gly Phe Ile Val Thr Leu Ser Leu Val Glu Leu Gly
 835 840 845

Leu Ala Asn Val Glu Gly Leu Ser Val Leu Arg Ser Phe Arg Leu Leu
 850 855 860

Arg Val Phe Lys Leu Ala Lys Ser Trp Pro Thr Leu Asn Met Leu Ile
 865 870 875 880

Lys Ile Ile Gly Asn Ser Val Gly Ala Leu Gly Asn Leu Thr Leu Val
 885 890 895

Leu Ala Ile Ile Val Phe Ile Phe Ala Val Val Gly Met Gln Leu Phe
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Gly Lys Ser Tyr Lys Asp Cys Val Cys Lys Ile Ala Ser Asp Cys Gln
 915 920 925

Leu Pro Arg Trp His Met Asn Asp Phe Phe His Ser Phe Leu Ile Val
 930 935 940

Phe Arg Val Leu Cys Gly Glu Trp Ile Glu Thr Met Trp Asp Cys Met
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Glu Val Ala Gly Gln Ala Met Cys Leu Thr Val Phe Met Met Val Met
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Val Ile Gly Asn Leu Val Val Leu Asn Leu Phe Leu Ala Leu Leu Leu
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Ser Ser Phe Ser Ala Asp Asn Leu Ala Ala Thr Asp Asp Asp Asn Glu
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SCN1APCT1.ST25.txt

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1070						1075					1080			
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1085						1090					1095			
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1115						1120					1125			
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SCN1APCT1.ST25.txt

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SCN1APCT1.ST25.txt

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Ser	Leu	Arg	His	Tyr	Tyr	Phe	Thr	Ile	Gly	Trp	Asn	Ile	Phe	Asp
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1610						1615					1620			
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1625						1630					1635			
Arg	Leu	Ala	Arg	Ile	Gly	Arg	Ile	Leu	Arg	Leu	Ile	Lys	Gly	Ala
1640						1645					1650			

SCN1APCT1.ST25.txt

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 1670 1675 1680
 Tyr Ala Ile Phe Gly Met Ser Asn Phe Ala Tyr Val Lys Arg Glu
 1685 1690 1695
 Val Gly Ile Asp Asp Met Phe Asn Phe Glu Thr Phe Gly Asn Ser
 1700 1705 1710
 Met Ile Cys Leu Phe Gln Ile Thr Thr Ser Ala Gly Trp Asp Gly
 1715 1720 1725
 Leu Leu Ala Pro Ile Leu Asn Ser Lys Pro Pro Asp Cys Asp Pro
 1730 1735 1740
 Asn Lys Val Asn Pro Gly Ser Ser Val Lys Gly Asp Cys Gly Asn
 1745 1750 1755
 Pro Ser Val Gly Ile Phe Phe Phe Val Ser Tyr Ile Ile Ile Ser
 1760 1765 1770
 Phe Leu Val Val Val Asn Met Tyr Ile Ala Val Ile Leu Glu Asn
 1775 1780 1785
 Phe Ser Val Ala Thr Glu Glu Ser Ala Glu Pro Leu Ser Glu Asp
 1790 1795 1800
 Asp Phe Glu Met Phe Tyr Glu Val Trp Glu Lys Phe Asp Pro Asp
 1805 1810 1815
 Ala Thr Gln Phe Met Glu Phe Glu Lys Leu Ser Gln Phe Ala Ala
 1820 1825 1830
 Ala Leu Glu Pro Pro Leu Asn Leu Pro Gln Pro Asn Lys Leu Gln
 1835 1840 1845
 Leu Ile Ala Met Asp Leu Pro Met Val Ser Gly Asp Arg Ile His
 1850 1855 1860

SCN1APCT1.ST25.txt

Cys Leu Asp Ile Leu Phe Ala Phe Thr Lys Arg Val Leu Gly Glu
1865 1870 1875

Ser Gly Glu Met Asp Ala Leu Arg Ile Gln Met Glu Glu Arg Phe
1880 1885 1890

Met Ala Ser Asn Pro Ser Lys Val Ser Tyr Gln Pro Ile Thr Thr
1895 1900 1905

Thr Leu Lys Arg Lys Gln Glu Glu Val Ser Ala Val Ile Ile Gln
1910 1915 1920

Arg Ala Tyr Arg Arg His Leu Leu Lys Arg Thr Val Lys Gln Ala
1925 1930 1935

Ser Phe Thr Tyr Asn Lys Asn Lys Ile Lys Gly Gly Ala Asn Leu
1940 1945 1950

Leu Ile Lys Glu Asp Met Ile Ile Asp Arg Ile Asn Glu Asn Ser
1955 1960 1965

Ile Thr Glu Lys Thr Asp Leu Thr Met Ser Thr Ala Ala Cys Pro
1970 1975 1980

Pro Ser Tyr Asp Arg Val Thr Lys Pro Ile Val Glu Lys His Glu
1985 1990 1995

Gln Glu Gly Lys Asp Glu Lys Ala Lys Gly Lys
2000 2005

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120

ggaatctgaa caattgcaac tgaaggcaca ttgttatcat ctgctctttg ggtgatgctg

180

SCN1APCT1.ST25.txt

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360
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1020

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1560
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1740
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SCN1APCT1.ST25.txt

1860

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1920

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1980

caagcctttt cagctttaga gggcgagcaa aggatgtggg atctgagaac gacttcgcag

2040

atgatgagca cagcaccttt gaggataacg agagccgtag agattccttg tttgtgcccc

2100

gacgacacgg agagagacgc aacagcaacc tgagtcagac cagtaggtca tcccggatgc

2160

tggcagtgtt tccagcgaat gggaagatgc acagcactgt ggattgcaat ggtgtggttt

2220

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2280

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2340

gaagggtcaag ttctttccac gtttccatgg actttctaga agatccttcc caaaggcaac

2400

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2460

agaaatgccc accctgttgg tataaatatt ccaacatatt cttaatctgg gactgttctc

2520

catattgggtt aaaagtgaac catgttgtca acctgggtgt gatggacca tttgttgacc

2580

tggccatcac catctgtatt gtcttaaata ctcttttcat ggccatggag cactatccaa

2640

tgacggacca tttcaataat gtgcttacag taggaaactt ggtttttact gggatcttta

SCN1APCT1.ST25.txt

2700

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2760

ggaatatctt tgacggtttt attgtgacgc ttagcctggt agaacttgga ctgcaccaatg

2820

tggaaggatt atctgttctc cgttcatttc gattgctgcg agttttcaag ttggcaaaat

2880

cttggccaac gttaaataatg ctaataaaga tcatcggaac ttccgtgggg gctctgggaa

2940

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3480

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3780

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4320

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6780

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SCN1APCT1.ST25.txt

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SCN1APCT1.ST25.txt

Met Glu Gln Thr Val Leu Val Pro Pro Gly Pro Asp Ser Phe Asn Phe
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 20 25 30
 Lys Ala Lys Asn Pro Lys Pro Asp Lys Lys Asp Asp Asp Glu Asn Gly
 35 40 45
 Pro Lys Pro Asn Ser Asp Leu Glu Ala Gly Lys Asn Leu Pro Phe Ile
 50 55 60
 Tyr Gly Asp Ile Pro Pro Glu Met Val Ser Glu Pro Leu Glu Asp Leu
 65 70 75 80
 Asp Pro Tyr Tyr Ile Asn Lys Lys Thr Phe Ile Val Leu Asn Lys Leu
 85 90 95
 Lys Ala Ile Phe Arg Phe Ser Ala Thr Ser Ala Leu Tyr Ile Leu Thr
 100 105 110
 Pro Phe Asn Pro Leu Arg Lys Ile Ala Ile Lys Ile Leu Val His Ser
 115 120 125
 Leu Phe Ser Met Leu Ile Met Cys Thr Ile Leu Thr Asn Cys Val Phe
 130 135 140
 Met Thr Met Ser Asn Pro Pro Asp Trp Thr Lys Asn Val Glu Tyr Thr
 145 150 155 160
 Phe Thr Gly Ile Tyr Thr Phe Glu Ser Leu Ile Lys Ile Ile Ala Arg
 165 170 175
 Gly Phe Cys Leu Glu Asp Phe Thr Phe Leu Arg Asp Pro Trp Asn Trp
 180 185 190
 Leu Asp Phe Thr Val Ile Thr Phe Ala Tyr Val Thr Glu Phe Val Asp
 195 200 205
 Leu Gly Asn Val Ser Ala Leu Arg Thr Phe Arg Val Leu Arg Ala Leu
 210 215 220

SCN1APCT1.ST25.txt

Lys Thr Ile Ser Val Ile Pro Gly Leu Lys Thr Ile Val Gly Ala Leu
 225 230 235 240

Ile Gln Ser Val Lys Lys Leu Ser Asp Val Met Ile Leu Thr Val Phe
 245 250 255

Cys Leu Ser Val Phe Ala Leu Ile Gly Leu Gln Leu Phe Met Gly Asn
 260 265 270

Leu Arg Asn Lys Cys Ile Gln Trp Pro Pro Thr Asn Ala Ser Leu Glu
 275 280 285

Glu His Ser Ile Glu Lys Asn Ile Thr Val Asn Tyr Asn Gly Thr Leu
 290 295 300

Ile Asn Glu Thr Val Phe Glu Phe Asp Trp Lys Ser Tyr Ile Gln Asp
 305 310 315 320

Ser Arg Tyr His Tyr Phe Leu Glu Gly Phe Leu Asp Ala Leu Leu Cys
 325 330 335

Gly Asn Ser Ser Asp Ala Gly Gln Cys Pro Glu Gly Tyr Met Cys Val
 340 345 350

Lys Ala Gly Arg Asn Pro Asn Tyr Gly Tyr Thr Ser Phe Asp Thr Phe
 355 360 365

Ser Trp Ala Phe Leu Ser Leu Phe Arg Leu Met Thr Gln Asp Phe Trp
 370 375 380

Glu Asn Leu Tyr Gln Leu Thr Leu Arg Ala Ala Gly Lys Thr Tyr Met
 385 390 395 400

Ile Phe Phe Val Leu Val Ile Phe Leu Gly Ser Phe Tyr Leu Ile Asn
 405 410 415

Leu Ile Leu Ala Val Val Ala Met Ala Tyr Glu Glu Gln Asn Gln Ala
 420 425 430

Thr Leu Glu Glu Ala Glu Gln Lys Glu Ala Glu Phe Gln Gln Met Ile

SCN1APCT1.ST25.txt
440

435

445

Glu Gln Leu Lys Lys Gln Gln Glu Ala Ala Gln Gln Ala Ala Thr Ala
 450 455 460

Thr Ala Ser Glu His Ser Arg Glu Pro Ser Ala Ala Gly Arg Leu Ser
 465 470 475 480

Asp Ser Ser Ser Glu Ala Ser Lys Leu Ser Ser Lys Ser Ala Lys Glu
 485 490 495

Arg Arg Asn Arg Arg Lys Lys Arg Lys Gln Lys Glu Gln Ser Gly Gly
 500 505 510

Glu Glu Lys Asp Glu Asp Glu Phe Gln Lys Ser Glu Ser Glu Asp Ser
 515 520 525

Ile Arg Arg Lys Gly Phe Arg Phe Ser Ile Glu Gly Asn Arg Leu Thr
 530 535 540

Tyr Glu Lys Arg Tyr Ser Ser Pro His Gln Ser Leu Leu Ser Ile Arg
 545 550 555 560

Gly Ser Leu Phe Ser Pro Arg Arg Asn Ser Arg Thr Ser Leu Phe Ser
 565 570 575

Phe Arg Gly Arg Ala Lys Asp Val Gly Ser Glu Asn Asp Phe Ala Asp
 580 585 590

Asp Glu His Ser Thr Phe Glu Asp Asn Glu Ser Arg Arg Asp Ser Leu
 595 600 605

Phe Val Pro Arg Arg His Gly Glu Arg Arg Asn Ser Asn Leu Ser Gln
 610 615 620

Thr Ser Arg Ser Ser Arg Met Leu Ala Val Phe Pro Ala Asn Gly Lys
 625 630 635 640

Met His Ser Thr Val Asp Cys Asn Gly Val Val Ser Leu Val Gly Gly
 645 650 655

SCN1APCT1.ST25.txt

Pro Ser Val Pro Thr Ser Pro Val Gly Gln Leu Leu Pro Glu Val Ile
 660 665 670

Ile Asp Lys Pro Ala Thr Asp Asp Asn Gly Thr Thr Thr Glu Thr Glu
 675 680 685

Met Arg Lys Arg Arg Ser Ser Ser Phe His Val Ser Met Asp Phe Leu
 690 695 700

Glu Asp Pro Ser Gln Arg Gln Arg Ala Met Ser Ile Ala Ser Ile Leu
 705 710 715 720

Thr Asn Thr Val Glu Glu Leu Glu Glu Ser Arg Gln Lys Cys Pro Pro
 725 730 735

Cys Trp Tyr Lys Phe Ser Asn Ile Phe Leu Ile Trp Asp Cys Ser Pro
 740 745 750

Tyr Trp Leu Lys Val Lys His Val Val Asn Leu Val Val Met Asp Pro
 755 760 765

Phe Val Asp Leu Ala Ile Thr Ile Cys Ile Val Leu Asn Thr Leu Phe
 770 775 780

Met Ala Met Glu His Tyr Pro Met Thr Asp His Phe Asn Asn Val Leu
 785 790 795 800

Thr Val Gly Asn Leu Val Phe Thr Gly Ile Phe Thr Ala Glu Met Phe
 805 810 815

Leu Lys Ile Ile Ala Met Asp Pro Tyr Tyr Tyr Phe Gln Glu Gly Trp
 820 825 830

Asn Ile Phe Asp Gly Phe Ile Val Thr Leu Ser Leu Val Glu Leu Gly
 835 840 845

Leu Ala Asn Val Glu Gly Leu Ser Val Leu Arg Ser Phe Arg Leu Leu
 850 855 860

Arg Val Phe Lys Leu Ala Lys Ser Trp Pro Thr Leu Asn Met Leu Ile
 865 870 875 880

SCN1APCT1.ST25.txt

Lys Ile Ile Gly Asn Ser Val Gly Ala Leu Gly Asn Leu Thr Leu Val
 885 890 895

Leu Ala Ile Ile Val Phe Ile Phe Ala Val Val Gly Met Gln Leu Phe
 900 905 910

Gly Lys Ser Tyr Lys Asp Cys Val Cys Lys Ile Ala Ser Asp Cys Gln
 915 920 925

Leu Pro Arg Trp His Met Asn Asp Phe Phe His Ser Phe Leu Ile Val
 930 935 940

Phe Arg Val Leu Cys Gly Glu Trp Ile Glu Thr Met Trp Asp Cys Met
 945 950 955 960

Glu Val Ala Gly Gln Ala Met Cys Leu Thr Val Phe Met Met Val Met
 965 970 975

Val Ile Gly Asn Leu Val Val Leu Asn Leu Phe Leu Ala Leu Leu Leu
 980 985 990

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Ala Tyr Val Lys Arg Lys Ile Tyr Glu Phe Ile Gln Gln Ser Phe
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Ile Arg Lys Gln Lys Ile Leu Asp Glu Ile Lys Pro Leu Asp Asp
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Leu Asn Asn Lys Lys Asp Ser Cys Met Ser Asn His Thr Thr Glu
 1055 1060 1065

Ile Gly Lys Asp Leu Asp Tyr Leu Lys Asp Val Asn Gly Thr Thr
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Ser Gly Ile Gly Thr Gly Ser Ser Val Glu Lys Tyr Ile Ile Asp
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SCN1APCT1.ST25.txt

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1880						1885					1890			
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1895						1900					1905			
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SCN1APCT1.ST25.txt

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1955 1960 1965

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Lys Ala Ile Phe Arg Phe Ser Ala Thr Ser Ala Leu Tyr Ile Leu Thr
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Pro Phe Asn Pro Leu Arg Lys Ile Ala Ile Lys Ile Leu Val His Ser
 115 120 125

Leu Phe Ser Met Leu Ile Met Cys Thr Ile Leu Thr Asn Cys Val Phe
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Met Thr Met Ser Asn Pro Pro Asp Trp Thr Lys Asn Val Glu Tyr Thr
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Gly Phe Cys Leu Glu Asp Phe Thr Phe Leu Arg Asp Pro Trp Asn Trp
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Leu Asp Phe Thr Val Ile Thr Phe Ala Tyr Val Thr Glu Phe Val Asp
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Leu Gly Asn Val Ser Ala Leu Arg Thr Phe Arg Val Leu Arg Ala Leu
 210 215 220

Lys Thr Ile Ser Val Ile Pro Gly Leu Lys Thr Ile Val Gly Ala Leu
 225 230 235 240

Ile Gln Ser Val Lys Lys Leu Ser Asp Val Met Ile Leu Thr Val Phe
 245 250 255

Cys Leu Ser Val Phe Ala Leu Ile Gly Leu Gln Leu Phe Met Gly Asn
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SCN1APCT1.ST25.txt

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 340 345 350

Lys Ala Gly Arg Asn Pro Asn Tyr Gly Tyr Thr Ser Phe Asp Thr Phe
 355 360 365

Ser Trp Ala Phe Leu Ser Leu Phe Arg Leu Met Thr Gln Asp Phe Trp
 370 375 380

Glu Asn Leu Tyr Gln Leu Thr Leu Arg Ala Ala Gly Lys Thr Tyr Met
 385 390 395 400

Ile Phe Phe Val Leu Val Ile Phe Leu Gly Ser Phe Tyr Leu Ile Asn
 405 410 415

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 485 490 495

Arg Arg Asn Arg Arg Lys Lys Arg Lys Gln Lys Glu Gln Ser Gly Gly

510

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SCN1APCT1.ST25.txt

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Ser Ser Phe Ser Ala Asp Asn Leu Ala Ala Thr Asp Asp Asp Asn Glu
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SCN1APCT1.ST25.txt

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SCN1APCT1.ST25.txt

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			20					25					30		

SCN1APCT1.ST25.txt

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 50 55 60
 Tyr Gly Asp Ile Pro Pro Glu Met Val Ser Glu Pro Leu Glu Asp Leu
 65 70 75 80
 Asp Pro Tyr Tyr Ile Asn Lys Lys Thr Phe Ile Val Leu Asn Lys Leu
 85 90 95
 Lys Ala Ile Phe Arg Phe Ser Ala Thr Ser Ala Leu Tyr Ile Leu Thr
 100 105 110
 Pro Phe Asn Pro Leu Arg Lys Ile Ala Ile Lys Ile Leu Val His Ser
 115 120 125
 Leu Phe Ser Met Leu Ile Met Cys Thr Ile Leu Thr Asn Cys Val Phe
 130 135 140
 Met Thr Met Ser Asn Pro Pro Asp Trp Thr Lys Asn Val Glu Tyr Thr
 145 150 155 160
 Phe Thr Gly Ile Tyr Thr Phe Glu Ser Leu Ile Lys Ile Ile Ala Arg
 165 170 175
 Gly Phe Cys Leu Glu Asp Phe Thr Phe Leu Arg Asp Pro Trp Asn Trp
 180 185 190
 Leu Asp Phe Thr Val Ile Thr Phe Ala Tyr Val Thr Glu Phe Val Asp
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 Leu Gly Asn Val Ser Ala Leu Arg Thr Phe Arg Val Leu Arg Ala Leu
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 Lys Thr Ile Ser Val Ile Pro Gly Leu Lys Thr Ile Val Gly Ala Leu
 225 230 235 240
 Ile Gln Ser Val Lys Lys Leu Ser Asp Val Met Ile Leu Thr Val Phe
 245 250 255

SCN1APCT1.ST25.txt

Cys Leu Ser Val Phe Ala Leu Ile Gly Leu Gln Leu Phe Met Gly Asn
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 Glu His Ser Ile Glu Lys Asn Ile Thr Val Asn Tyr Asn Gly Thr Leu
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 Ile Asn Glu Thr Val Phe Glu Phe Asp Trp Lys Ser Tyr Ile Gln Asp
 305 310 315 320
 Ser Arg Tyr His Tyr Phe Leu Glu Gly Phe Leu Asp Ala Leu Leu Cys
 325 330 335
 Gly Asn Ser Ser Asp Ala Gly Gln Cys Pro Glu Gly Tyr Met Cys Val
 340 345 350
 Lys Ala Gly Arg Asn Pro Asn Tyr Gly Tyr Thr Ser Phe Asp Thr Phe
 355 360 365
 Ser Trp Ala Phe Leu Ser Leu Phe Arg Leu Met Thr Gln Asp Phe Trp
 370 375 380
 Glu Asn Leu Tyr Gln Leu Thr Leu Arg Ala Ala Gly Lys Thr Tyr Met
 385 390 395 400
 Ile Phe Phe Val Leu Val Ile Phe Leu Gly Ser Phe Tyr Leu Ile Asn
 405 410 415
 Leu Ile Leu Ala Val Val Ala Met Ala Tyr Glu Glu Gln Asn Gln Ala
 420 425 430
 Thr Leu Glu Glu Ala Glu Gln Lys Glu Ala Glu Phe Gln Gln Met Ile
 435 440 445
 Glu Gln Leu Lys Lys Gln Gln Glu Ala Ala Gln Gln Ala Ala Thr Ala
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SCN1APCT1.ST25.txt

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Glu Glu Lys Asp Glu Asp Glu Phe Gln Lys Ser Glu Ser Glu Asp Ser
      515                               520                               525

Ile Arg Arg Lys Gly Phe Arg Phe Ser Ile Glu Gly Asn Arg Leu Thr
      530                               535                               540

Tyr Glu Lys Arg Tyr Ser Ser Pro His Gln Ser Leu Leu Ser Ile Arg
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Gly Ser Leu Phe Ser Pro Arg Arg Asn Ser Arg Thr Ser Leu Phe Ser
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Asp Glu His Ser Thr Phe Glu Asp Asn Glu Ser Arg Arg Asp Ser Leu
      595                               600                               605

Phe Val Pro Arg Arg His Gly Glu Arg Arg Asn Ser Asn Leu Ser Gln
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Thr Ser Arg Ser Ser Arg Met Leu Ala Val Phe Pro Ala Asn Gly Lys
      625                               630                               635                               640

Met His Ser Thr Val Asp Cys Asn Gly Val Val Ser Leu Val Gly Gly
      645                               650                               655

Pro Ser Val Pro Thr Ser Pro Val Gly Gln Leu Leu Pro Glu Val Ile
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Ile Asp Lys Pro Ala Thr Asp Asp Asn Gly Thr Thr Thr Glu Thr Glu
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SCN1APCT1.ST25.txt

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725 730 735

Cys Trp Tyr Lys Phe Ser Asn Ile Phe Leu Ile Trp Asp Cys Ser Pro
740 745 750

Tyr Trp Leu Lys Val Lys His Val Val Asn Leu Val Val Met Asp Pro
755 760 765

Phe Val Asp Leu Ala Ile Thr Ile Cys Ile Val Leu Asn Thr Leu Phe
770 775 780

Met Ala Met Glu His Tyr Pro Met Thr Asp His Phe Asn Asn Val Leu
785 790 795 800

Thr Val Gly Asn Leu Val Phe Thr Gly Ile Phe Thr Ala Glu Met Phe
805 810 815

Leu Lys Ile Ile Ala Met Asp Pro Tyr Tyr Tyr Phe Gln Glu Gly Trp
820 825 830

Asn Ile Phe Asp Gly Phe Ile Val Thr Leu Ser Leu Val Glu Leu Gly
835 840 845

Leu Ala Asn Val Glu Gly Leu Ser Val Leu Arg Ser Phe Arg Leu Leu
850 855 860

Arg Val Phe Lys Leu Ala Lys Ser Trp Pro Thr Leu Asn Met Leu Ile
865 870 875 880

Lys Ile Ile Gly Asn Ser Val Gly Ala Leu Gly Asn Leu Thr Leu Val
885 890 895

Leu Ala Ile Ile Val Phe Ile Phe Ala Val Val Gly Met Gln Leu Phe
900 905 910

SCN1APCT1.ST25.txt

Gly Lys Ser Tyr Lys Asp Cys Val Cys Lys Ile Ala Ser Asp Cys Gln
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 930 935 940
 Phe Arg Val Leu Cys Gly Glu Trp Ile Glu Thr Met Trp Asp Cys Met
 945 950 955 960
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 965 970 975
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 980 985 990
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 Ala Tyr Val Lys Arg Lys Ile Tyr Glu Phe Ile Gln Gln Ser Phe
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 1055 1060 1065
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 1070 1075 1080
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SCN1APCT1.ST25.txt

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Lys	Leu	Asn	Glu	Ser	Ser	Ser	Ser	Ser	Glu	Gly	Ser	Thr	Val	Asp
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1160						1165					1170			
Thr	Leu	Glu	Pro	Glu	Ala	Cys	Phe	Thr	Glu	Gly	Cys	Val	Gln	Arg
1175						1180					1185			
Phe	Lys	Cys	Cys	Gln	Ile	Asn	Val	Glu	Glu	Gly	Arg	Gly	Lys	Gln
1190						1195					1200			
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1280						1285					1290			
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1310						1315					1320			
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Page 97

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SCN1APCT1.ST25.txt

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SCN1APCT1.ST25.txt

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1800

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1860

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1920

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1980

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2040

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2100

gacgacacgg agagagacgc aacagcaacc tgagtcagac cagtaggtca tcccggatgc
2160

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SCN1APCT1.ST25.txt

2220

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2280

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2340

gaagggtcaag ttctttccac gtttccatgg actttctaga agatccttcc caaaggcaac

2400

gagcaatgag tatagccagc attctaacia atacagtaga agaacttgaa gaatccaggc

2460

agaaatgcc accctggttg tataaatatt ccaacatatt cttaatctgg gactgttctc

2520

catattgggtt aaaagtgaia catgttgtca acctggttgt gatggacca tttgttgacc

2580

tggccatcac catctgtatt gtcttaaata ctcttttcat ggccatggag cactatccaa

2640

tgacggacca tttcaataat gtgcttacag taggaaactt ggttttcact gggatcttta

2700

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2760

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2820

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2880

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2940

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SCN1APCT1.ST25.txt

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3240
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3300
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3360
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3840

SCN1APCT1.ST25.txt

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4680

SCN1APCT1.ST25.txt

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4740

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4800

aaaaattagg atcgaaaaaa ccgcaaaagc ctatacctcg accaggaaac aaatttcaag
4860

gaatggtctt tgacttcgta accagacaag tttttgacat aagcatcatg attctcatct
4920

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4980

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SCN1APCT1.ST25.txt

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SCN1APCT1.ST25.txt

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SCN1APCT1.ST25.txt

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SCN1APCT1.ST25.txt

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8280

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Phe	Thr	Arg	Glu	Ser	Leu	Ala	Ala	Ile	Glu	Arg	Arg	Ile	Ala	Glu	Glu
			20					25					30		

Lys	Ala	Lys	Asn	Pro	Lys	Pro	Asp	Lys	Lys	Asp	Asp	Asp	Glu	Asn	Gly
		35					40					45			

Pro	Lys	Pro	Asn	Ser	Asp	Leu	Glu	Ala	Gly	Lys	Asn	Leu	Pro	Phe	Ile
	50					55					60				

Tyr	Gly	Asp	Ile	Pro	Pro	Glu	Met	Val	Ser	Glu	Pro	Leu	Glu	Asp	Leu
65					70					75					80

Asp	Pro	Tyr	Tyr	Ile	Asn	Lys	Lys	Thr	Phe	Ile	Val	Leu	Asn	Lys	Leu
				85					90					95	

SCN1APCT1.ST25.txt

Lys Ala Ile Phe Arg Phe Ser Ala Thr Ser Ala Leu Tyr Ile Leu Thr
 100 105 110
 Pro Phe Asn Pro Leu Arg Lys Ile Ala Ile Lys Ile Leu Val His Ser
 115 120 125
 Leu Phe Ser Met Leu Ile Met Cys Thr Ile Leu Thr Asn Cys Val Phe
 130 135 140
 Met Thr Met Ser Asn Pro Pro Asp Trp Thr Lys Asn Val Glu Tyr Thr
 145 150 155 160
 Phe Thr Gly Ile Tyr Thr Phe Glu Ser Leu Ile Lys Ile Ile Ala Arg
 165 170 175
 Gly Phe Cys Leu Glu Asp Phe Thr Phe Leu Arg Asp Pro Trp Asn Trp
 180 185 190
 Leu Asp Phe Thr Val Ile Thr Phe Ala Tyr Val Thr Glu Phe Val Asp
 195 200 205
 Leu Gly Asn Val Ser Ala Leu Arg Thr Phe Arg Val Leu Arg Ala Leu
 210 215 220
 Lys Thr Ile Ser Val Ile Pro Gly Leu Lys Thr Ile Val Gly Ala Leu
 225 230 235 240
 Ile Gln Ser Val Lys Lys Leu Ser Asp Val Met Ile Leu Thr Val Phe
 245 250 255
 Cys Leu Ser Val Phe Ala Leu Ile Gly Leu Gln Leu Phe Met Gly Asn
 260 265 270
 Leu Arg Asn Lys Cys Ile Gln Trp Pro Pro Thr Asn Ala Ser Leu Glu
 275 280 285
 Glu His Ser Ile Glu Lys Asn Ile Thr Val Asn Tyr Asn Gly Thr Leu
 290 295 300
 Ile Asn Glu Thr Val Phe Glu Phe Asp Trp Lys Ser Tyr Ile Gln Asp
 305 310 315 320

SCN1APCT1.ST25.txt

Ser Arg Tyr His Tyr Phe Leu Glu Gly Phe Leu Asp Ala Leu Leu Cys
 325 330 335
 Gly Asn Ser Ser Asp Ala Gly Gln Cys Pro Glu Gly Tyr Met Cys Val
 340 345 350
 Lys Ala Gly Arg Asn Pro Asn Tyr Gly Tyr Thr Ser Phe Asp Thr Phe
 355 360 365
 Ser Trp Ala Phe Leu Ser Leu Phe Arg Leu Met Thr Gln Asp Phe Trp
 370 375 380
 Glu Asn Leu Tyr Gln Leu Thr Leu Arg Ala Ala Gly Lys Thr Tyr Met
 385 390 395 400
 Ile Phe Phe Val Leu Val Ile Phe Leu Gly Ser Phe Tyr Leu Ile Asn
 405 410 415
 Leu Ile Leu Ala Val Val Ala Met Ala Tyr Glu Glu Gln Asn Gln Ala
 420 425 430
 Thr Leu Glu Glu Ala Glu Gln Lys Glu Ala Glu Phe Gln Gln Met Ile
 435 440 445
 Glu Gln Leu Lys Lys Gln Gln Glu Ala Ala Gln Gln Ala Ala Thr Ala
 450 455 460
 Thr Ala Ser Glu His Ser Arg Glu Pro Ser Ala Ala Gly Arg Leu Ser
 465 470 475 480
 Asp Ser Ser Ser Glu Ala Ser Lys Leu Ser Ser Lys Ser Ala Lys Glu
 485 490 495
 Arg Arg Asn Arg Arg Lys Lys Arg Lys Gln Lys Glu Gln Ser Gly Gly
 500 505 510
 Glu Glu Lys Asp Glu Asp Glu Phe Gln Lys Ser Glu Ser Glu Asp Ser
 515 520 525
 Ile Arg Arg Lys Gly Phe Arg Phe Ser Ile Glu Gly Asn Arg Leu Thr

SCN1APCT1.ST25.txt

530

535

540

Tyr Glu Lys Arg Tyr Ser Ser Pro His Gln Ser Leu Leu Ser Ile Arg
 545 550 555 560

Gly Ser Leu Phe Ser Pro Arg Arg Asn Ser Arg Thr Ser Leu Phe Ser
 565 570 575

Phe Arg Gly Arg Ala Lys Asp Val Gly Ser Glu Asn Asp Phe Ala Asp
 580 585 590

Asp Glu His Ser Thr Phe Glu Asp Asn Glu Ser Arg Arg Asp Ser Leu
 595 600 605

Phe Val Pro Arg Arg His Gly Glu Arg Arg Asn Ser Asn Leu Ser Gln
 610 615 620

Thr Ser Arg Ser Ser Arg Met Leu Ala Val Phe Pro Ala Asn Gly Lys
 625 630 635 640

Met His Ser Thr Val Asp Cys Asn Gly Val Val Ser Leu Val Gly Gly
 645 650 655

Pro Ser Val Pro Thr Ser Pro Val Gly Gln Leu Leu Pro Glu Val Ile
 660 665 670

Ile Asp Lys Pro Ala Thr Asp Asp Asn Gly Thr Thr Thr Glu Thr Glu
 675 680 685

Met Arg Lys Arg Arg Ser Ser Ser Phe His Val Ser Met Asp Phe Leu
 690 695 700

Glu Asp Pro Ser Gln Arg Gln Arg Ala Met Ser Ile Ala Ser Ile Leu
 705 710 715 720

Thr Asn Thr Val Glu Glu Leu Glu Glu Ser Arg Gln Lys Cys Pro Pro
 725 730 735

Cys Trp Tyr Lys Phe Ser Asn Ile Phe Leu Ile Trp Asp Cys Ser Pro
 740 745 750

SCN1APCT1.ST25.txt

Tyr Trp Leu Lys Val Lys His Val Val Asn Leu Val Val Met Asp Pro
 755 760 765

Phe Val Asp Leu Ala Ile Thr Ile Cys Ile Val Leu Asn Thr Leu Phe
 770 775 780

Met Ala Met Glu His Tyr Pro Met Thr Asp His Phe Asn Asn Val Leu
 785 790 795 800

Thr Val Gly Asn Leu Val Phe Thr Gly Ile Phe Thr Ala Glu Met Phe
 805 810 815

Leu Lys Ile Ile Ala Met Asp Pro Tyr Tyr Tyr Phe Gln Glu Gly Trp
 820 825 830

Asn Ile Phe Asp Gly Phe Ile Val Thr Leu Ser Leu Val Glu Leu Gly
 835 840 845

Leu Ala Asn Val Glu Gly Leu Ser Val Leu Arg Ser Phe Arg Leu Leu
 850 855 860

Arg Val Phe Lys Leu Ala Lys Ser Trp Pro Thr Leu Asn Met Leu Ile
 865 870 875 880

Lys Ile Ile Gly Asn Ser Val Gly Ala Leu Gly Asn Leu Thr Leu Val
 885 890 895

Leu Ala Ile Ile Val Phe Ile Phe Ala Val Val Gly Met Gln Leu Phe
 900 905 910

Gly Lys Ser Tyr Lys Asp Cys Val Cys Lys Ile Ala Ser Asp Cys Gln
 915 920 925

Leu Pro Arg Trp His Met Asn Asp Phe Phe His Ser Phe Leu Ile Val
 930 935 940

Phe Arg Val Leu Cys Gly Glu Trp Ile Glu Thr Met Trp Asp Cys Met
 945 950 955 960

Glu Val Ala Gly Gln Ala Met Cys Leu Thr Val Phe Met Met Val Met
 965 970 975

SCN1APCT1.ST25.txt

Val Ile Gly Asn Leu Val Val Leu Asn Leu Phe Leu Ala Leu Leu Leu
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Ser Ser Phe Ser Ala Asp Asn Leu Ala Ala Thr Asp Asp Asp Asn Glu
 995 1000 1005

Met Asn Asn Leu Gln Ile Ala Val Asp Arg Met His Lys Gly Val
 1010 1015 1020

Ala Tyr Val Lys Arg Lys Ile Tyr Glu Phe Ile Gln Gln Ser Phe
 1025 1030 1035

Ile Arg Lys Gln Lys Ile Leu Asp Glu Ile Lys Pro Leu Asp Asp
 1040 1045 1050

Leu Asn Asn Lys Lys Asp Ser Cys Met Ser Asn His Thr Thr Glu
 1055 1060 1065

Ile Gly Lys Asp Leu Asp Tyr Leu Lys Asp Val Asn Gly Thr Thr
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 1085 1090 1095

Glu Ser Asp Tyr Met Ser Phe Ile Asn Asn Pro Ser Leu Thr Val
 1100 1105 1110

Thr Val Pro Ile Ala Val Gly Glu Ser Asp Phe Glu Asn Leu Asn
 1115 1120 1125

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 1130 1135 1140

Lys Leu Asn Glu Ser Ser Ser Ser Ser Glu Gly Ser Thr Val Asp
 1145 1150 1155

Ile Gly Ala Pro Val Glu Glu Gln Pro Val Val Glu Pro Glu Glu
 1160 1165 1170

Thr Leu Glu Pro Glu Ala Cys Phe Thr Glu Gly Cys Val Gln Arg
 1175 1180 1185

SCN1APCT1.ST25.txt

Phe Lys Cys Cys Gln Ile Asn Val Glu Glu Gly Arg Gly Lys Gln
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 1205 1210 1215
 Trp Phe Glu Thr Phe Ile Val Phe Met Ile Leu Leu Ser Ser Gly
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 Ala Leu Ala Phe Glu Asp Ile Tyr Ile Asp Gln Arg Lys Thr Ile
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 1370 1375 1380
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SCN1APCT1.ST25.txt

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1415		1420		1425
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Ser Arg Asn Val Glu Leu Gln Pro Lys Tyr Glu Lys Ser Leu Tyr				
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Thr Leu Asn Leu Phe Ile Gly Val Ile Ile Asp Asn Phe Asn Gln				
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Gln Lys Lys Lys Phe Gly Gly Gln Asp Ile Phe Met Thr Glu Glu				
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Gln Lys Lys Tyr Tyr Asn Ala Met Lys Lys Leu Gly Ser Lys Lys				
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Pro Gln Lys Pro Ile Pro Arg Pro Gly Asn Lys Phe Gln Gly Met				
1520		1525		1530
Val Phe Asp Phe Val Thr Arg Gln Val Phe Asp Ile Ser Ile Met				
1535		1540		1545
Ile Leu Ile Cys Leu Asn Met Val Thr Met Met Val Glu Thr Asp				
1550		1555		1560
Asp Gln Ser Glu Tyr Val Thr Thr Ile Leu Ser Arg Ile Asn Leu				
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Val Phe Ile Val Leu Phe Thr Gly Glu Cys Val Leu Lys Leu Ile				
1580		1585		1590

SCN1APCT1.ST25.txt

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1610						1615					1620			
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1625						1630					1635			
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1640						1645					1650			
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1670						1675					1680			
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1685						1690					1695			
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1715						1720					1725			
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1730						1735					1740			
Asn	Lys	Val	Asn	Pro	Gly	Ser	Ser	Val	Lys	Gly	Asp	Cys	Gly	Asn
1745						1750					1755			
Pro	Ser	Val	Gly	Ile	Phe	Phe	Phe	Val	Ser	Tyr	Ile	Ile	Ile	Ser
1760						1765					1770			
Phe	Leu	Val	Val	Val	Asn	Met	Tyr	Ile	Ala	Val	Ile	Leu	Glu	Asn
1775						1780					1785			
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SCN1APCT1.ST25.txt

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 1835 1840 1845
 Leu Ile Ala Met Asp Leu Pro Met Val Ser Gly Asp Arg Ile His
 1850 1855 1860
 Cys Leu Asp Ile Leu Phe Ala Phe Thr Lys Arg Val Leu Gly Glu
 1865 1870 1875
 Ser Gly Glu Met Asp Ala Leu Arg Ile Gln Met Glu Glu Arg Phe
 1880 1885 1890
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 1895 1900 1905
 Thr Leu Lys Arg Lys Gln Glu Glu Val Ser Ala Val Ile Ile Gln
 1910 1915 1920
 Arg Ala Tyr Arg Gly His Leu Leu Lys Arg Thr Val Lys Gln Ala
 1925 1930 1935
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 1940 1945 1950
 Leu Ile Lys Glu Asp Met Ile Ile Asp Arg Ile Asn Glu Asn Ser
 1955 1960 1965
 Ile Thr Glu Lys Thr Asp Leu Thr Met Ser Thr Ala Ala Cys Pro
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